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RAPID COMMUNICATION / COMMUNICATION RAPIDE

Allee effects and compensatory population dynamics within a stock complex

Kenneth T. Frank and David Brickman

Abstract: Sparked by the observation that (*i*) many collapsed stocks have failed to recover despite the apparent prevalence of compensatory population dynamics and (*ii*) many stocks have a complex structure, we devised a simulation model illustrating that the underlying behavior of substocks is masked when the data are aggregated and evaluated at the scale of the management unit. The model consisted of several substocks within a stock complex, each having its own stock and recruitment (*S–R*) relationship with Allee effects. Incorporation of Allee effects into the *S–R* relationship was achieved by specifying a critical spawning stock biomass threshold below which no recruitment occurred. The simulation model revealed that it was possible for the aggregate *S–R* relationship to appear compensatory, even though no substock exhibited this behavior. If the conclusions we draw from our modeling are correct, biological reference points developed from aggregated data from multiple substocks and used in conventional fisheries management and stock assessment models are likely to be inaccurate and possibly nonconservative. More research in support of the delineation of substock structure, biophysical modeling, and metapopulation theory is advocated.

Résumé : Troublés d'observer que (*i*) de nombreux stocks effondrés n'ont pas réussi à se rétablir malgré la prévalence apparente d'une dynamique démographique compensatoire, et que (*ii*) de nombreux stocks ont une structure complexe, nous avons conçu un modèle de simulation montrant que le comportement sous-jacent des sous-stocks est masqué quand les données sont agrégées et évaluées à l'échelle de l'unité de gestion. Le modèle se composait de plusieurs sous-stocks au sein d'un complexe de stocks, dont chacun présentait sa propre relation stock-recrutement (*S–R*) avec des effets d'Allee. Pour intégrer les effets d'Allee à la relation *S–R*, nous avons spécifié un seuil critique de biomasse du stock reproducteur au-dessous duquel le recrutement était nul. Le modèle de simulation a révélé qu'il était possible que la relation *S–R* agrégée semble avoir un caractère compensatoire alors même qu'aucun des sous-stocks n'était dans ce cas. Si les conclusions que nous tirons de notre modèle sont correctes, les points de référence biologique établis à partir des données agrégées concernant des sous-stocks multiples, qui servent dans les modèles classiques de gestion des pêches et d'évaluation des stocks, sont vraisemblablement inexacts et ne respectent peut-être pas les objectifs de la conservation. Nous préconisons donc d'intensifier les recherches visant la détermination de la structure des sous-stocks, la modélisation biophysique et la théorie des métapopulations.

[Traduit par la Rédaction]

Introduction

Stock collapses worldwide attest to the fact that fisheries management has not been successful in controlling exploitation rates. In the Canadian Atlantic region, several cod (*Gadus morhua*) stocks have been severely overfished and reductions in spawning biomass range from 77 to 99% of the historical maximum (Myers et al. 1997*a*). To counteract these declines, fishing moratoria were imposed in all affected management units based on the implicit assumption that the effects of overfishing are reversible.

Myers et al. (1997*b*) predicted an annual growth rate of northern cod to be 19% following its collapse in 1992. Northern cod should have more than tripled in size after 7 years of fishing moratorium yet, to date, there are no significant indications of recovery (Shelton and Healey 1999). Other Canadian Atlantic cod stocks are equally notable for their failure to recover following collapses in the early 1990s, including the eastern Scotian Shelf, southern Grand Banks, and southern Gulf of St. Lawrence stocks. In his review of the collapse of 10 major fisheries for small pelagic marine species, Beverton (1990) noted that only one stock has fully regained its original size and that Icelandic spring-spawning herring (*Clupea harengus harengus*) has failed to reappear after 20 years. On the other hand, several depleted stocks for which reductions in fishing mortality have been effected have in fact recovered, albeit slowly. Al-

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Table 1. Visual display of the conceptual model showing the time course of sampling stock and recruitment data from each of the eight substocks within a hypothetical management unit.

Year	Substock 1	Substock 2	Substock 3	Substock 4	Substock 5	Substock 6	Substock 7	Substock 8	Total
1	S_1, R_1	S_2, R_2	S_3, R_3	S_4, R_4	S_5, R_5	S_6, R_6	S_7, R_7	S_8, R_8	S_{tot}, R_{tot}
2	" "	" "	" "	" "	" "	" "	" "	" "	" "
3	" "	" "	" "	" "	" "	" "	" "	" "	" "
4	" "	" "	" "	" "	" "	" "	" "	" "	" "
5	S_{crit}	" "	" "	" "	" "	" "	" "	" "	" "
6	"	" "	" "	" "	" "	" "	" "	" "	" "
7	"	S_{crit}	" "	" "	" "	" "	" "	" "	" "
8	"	"	" "	" "	" "	" "	" "	" "	" "
9	S_{ext}	"	" "	" "	" "	" "	" "	" "	" "
10		"	" "	" "	" "	" "	" "	" "	" "
11		S_{ext}	" "	" "	" "	" "	" "	" "	" "
.			S_{crit}	" "	" "	" "	" "	" "	" "
.			"	" "	" "	" "	" "	" "	" "
.			S_{ext}	" "	" "	" "	" "	" "	" "
43				" "	" "	" "	" "	" "	" "
44				S_{crit}	" "	" "	" "	" "	" "
45				"	" "	" "	" "	" "	" "
46				"	S_{crit}	" "	" "	" "	" "
47				"	"	" "	" "	" "	" "
48				S_{ext}	"	S_{crit}	" "	" "	" "
49					"	"	" "	" "	" "
50					S_{ext}	"	S_{crit}	" "	" "
51						"	"	" "	" "

Note: The thresholds (S_{crit}, the critical spawning stock biomass level below which no recruitment occurs, and S_{ext}, the extinction of the spawning stock) are reached at different times because of the different starting values of S. At the end of the simulation (run length equal 51 years), five of the eight substocks are extinct. The final column in the table shows the sum of the stock and recruitment data from all of the substocks, the level of data aggregation normally available for analysis of stock and recruitment relationships. Please note that the simulation did not run precisely in the manner depicted but is presented for illustrative purposes only.

though the time scales for recovery of collapsed stocks may be difficult to predict, it is clear that stocks that decline the most are the slowest to recover.

Why is it that so many collapsed stocks have failed to recover? A recent study suggests that the most frequent form of fish population regulation is compensatory population dynamics (Myers et al. 1995). The Ricker and Beverton and Holt stock (SSB) and recruitment (R) models rely on a compensatory response — increasing per capita reproductive success (R/SSB) with decreasing stock size. In other words, increasing prerecruit survival compensates for reductions in egg production associated with decreasing SSB. This leads to the assumption that when fishing mortality is eliminated from an overfished stock it should recover to a stable equilibrium at relatively high levels of SSB. However, the failure of collapsed stocks to recover suggests that other mechanisms are influencing the production dynamics at low population levels.

An infrequently encountered population dynamic model, but one consistent with the lack of recovery of collapsed stocks, is depensatory population dynamics. In this model, juvenile survival *decreases* at low stock levels and a second, lower equilibrium point exists that is unstable. If the stock is reduced to levels below this secondary equilibrium, it will collapse. A number of mechanisms may explain how depensation might occur and nearly all concentrate on predation. Recent studies (e.g., Shelton and Healey 1999) have suggested that this alternative form of population regulation may not be as infrequent as previously suggested by Myers et al. (1995). The former authors argue that depensation is difficult to detect because of limited and variable data associated with low spawning stock sizes and it would be prudent to allow for the possibility of depensation in fish population dynamics. Oddly, a major assumption associated with both the compensatory and depensatory stock and recruitment relationships is that the curve should pass through the origin, despite the lack of data at low population levels.

A more widespread, ecological phenomena associated with collapsed populations are the so-called Allee effects (see Myers et al. 1995 for reference to Allee). In contrast to depensation, the mechanisms underlying Allee effects are physiological and behavioral. These result in decreased reproduction at low population sizes. Examples of Allee effects include difficulty in finding a mate, a breakdown in social structure and migration patterns, difficulty in fending off predators or competitors, and a requirement for exceeding a critical population size before food resources can be properly exploited. Species for which Allee effects have been demonstrated or hypothesized include Pacific sardine, Downs herring, scallops, abalone, sea urchins, giant clams, muskrats, flour beetles, sheep ticks, and passenger pigeons. No S–R relationship incorporating Allee effects has ever been devised. Such a model would have one outstanding feature — a non-zero intercept defining a species-specific SSB threshold associated with absolute recruitment failure. In practice, such a relationship would probably be statistically indistinguishable from a depensatory S–R relationship.

We remain faced with two difficulties: (*i*) how to explain

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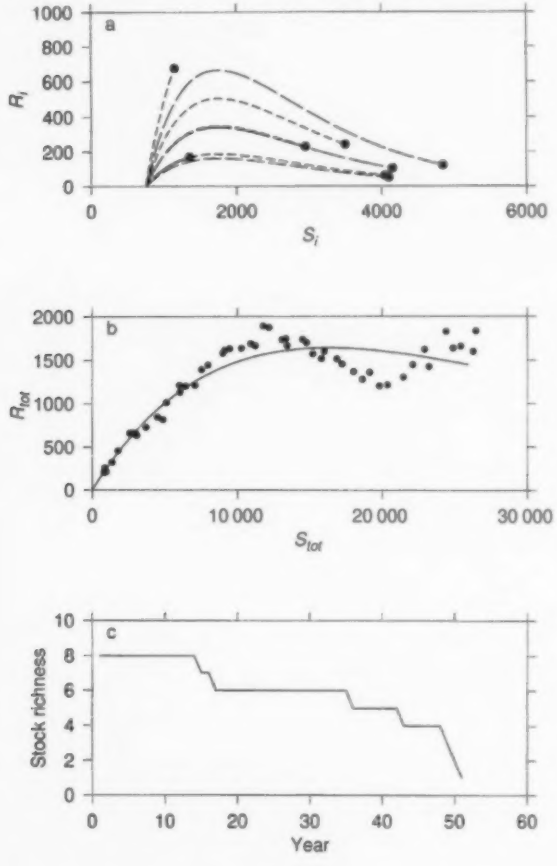
Fig. 1. (*a*) The assumed stock and recruitment relationships for each of the eight substocks contained within a hypothetical management unit. The closed circle on each curve represents the randomly selected starting point for the sampling (or incremental depletion) of each substock. Although each of the stock and recruitment relationships have varying amplitudes, model runs using equal amplitudes yield nearly identical results. (*b*) Recruitment and spawning stock data from the hypothetical management unit (equivalent to R_{tot} and S_{tot} from Table 1, respectively) generated during the 51 years. The best fitting curve to the data is a Ricker model passing through the origin. (*c*) The temporal trend in stock richness (number of substocks).



the fact that most *S–R* data from collapsed stocks conform to compensatory *S–R* dynamics but many do not recover and (*ii*) given the lack of *S–R* data at low population sizes, how to determine whether or not Allee effects are operative as they would be consistent with failed recovery. We will attempt to answer the first of these questions in two ways: first, by reminding ourselves that stocks within management units are generally a heterogeneous mix of smaller substocks and, second, through simulation modeling.

Stock complexes

It is widely recognized that many stocks have a complex structure, consisting of a heterogeneous mix of small substocks within a given management unit. Numerous studies using meristic and morphometric techniques, tagging, otolith chemistry, and genetic methods have supported this fact. This wealth of evidence has led to the conclusion that there are few fisheries in the world that are truly single-stock fisheries; the potential for complex stock structure is nearly always present. Unfortunately, fisheries management has generally failed to protect or maintain this complexity (Sinclair et al. 1996). Before the collapse of Northern cod, several substocks were present in the management unit. After the collapse of this stock, only one or two substocks remained (deYoung and Rose 1993). Both North Sea herring and eastern Scotian Shelf cod showed similar erosion of stock structure prior to collapse (see Cury et al. (2000) and Sinclair et al. (1996), respectively). The other feature common to these three examples is that the *S–R* data exhibit strong compensatory dynamics at the scale of the management unit (Myers et al. 1995). However, compensatory population dynamics are incapable of addressing the erosion of substock structure. What if Allee effects are indeed operative? Can they be shown to account for the dynamics of individual substocks in a manner consistent with the compensatory dynamics at the management unit scale?

Simulation model

Consider a stock complex consisting of numerous substocks existing within a single management unit, with each substock exhibiting a unique *S–R* relationship characterized by an Allee effect (i.e., their *S–R* relationships do not intersect the origin, but rather exhibit zero recruitment at some minimum stock size). The simulation was, in essence, a sampling experiment in which each of the underlying substocks was "fished down" to generate annual estimates of SSB and *R*. The depletion of the individual substocks began at different starting values of SSB, resulting in the gradual elimination of the substocks through time. The question is whether the aggregate (all substocks combined) *S–R* relationship would exhibit one of the properties of compensatory population dynamics, namely that the curve passes through the origin.

Methods

The simulated stock complex consisted of a management unit made up of $M = 8$ substocks, each having a *S–R* relationship that incorporates the Allee effect:

$$R_i(S) = A_i(S - S1)\exp(-B(S - S1))$$

where i is the substock, R is recruitment, S is some measure of the spawning stock, $S1 = 750$ is the offset from the origin representing the zero recruitment value (Allee effect), and A and B are constants. To simulate distinct substocks with differing initial size, we set (see table 11.5 of Ricker 1975) $B = 0.001$ and $A_i = 2.7180_i$ (where θ_i is a random number between 0 and 1) and chose S_0, the initial S, at random.

Subsequent values for S decreased by ΔS and increased by some fraction of recruitment ΔR_i, where ΔR_i equals $\theta_i R_i$, and ΔS was chosen as a fraction of the maximum S_0 (i.e., maximum initial substock size) such that it was likely that after the $N = 51$-year run length, there would be some stocks remaining. If $R_i(S) \leq 0$, then R_i was set to zero. If $S_i \leq 0$, then the substock was considered extinct. For each year, the total S and R was calculated (S_{tot}, R_{tot}) summed over the M substocks, and the number of nonextinct stocks was noted. The unidirectional decrement of the individual spawning

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stocks was justified on the basis that most fisheries data exhibit continuously increasing fishing effort and declining catch per unit effort. Because S is never known precisely, an uncertainty was allowed for $R(S)$

$$R(S) = R(S + \mathrm{d}S)$$

where $\mathrm{d}S = \theta_2 S$, i.e., S was set to $S = S + \mathrm{d}S$, $R(S')$ was computed and assigned to $R(S)$. Here, θ_2 is a random number between –0.1 and 0.1. A conceptual form of the simulation model is presented in Table 1.

Model results

The individual $R(S)$ curves are illustrated in Fig. 1*a*. The corresponding R_{tot}–S_{tot} relationship and the best fit to these data using a typical Ricker-type compensatory model are illustrated in Fig. 1*b*. The temporal change in stock richness (the decay in substock numbers with time) is illustrated in Fig. 1*c*.

The simulation produced a monotonic decline in spawning stock biomass (S_{tot}) and a cascading decline in recruitment (R_{tot}) at the management unit scale. The result (Fig. 1*b*) shows that it is *possible* for the aggregate S–R relationship to appear compensatory, even though no substocks exhibited this behavior. This has two consequences: (*i*) it falsely conforms to the assumption that relaxation of fishing will permit recovery, and (*ii*) it prevents recognition of the critical state of substocks. Not recognizing the substock structure masks the extinction of substocks as a general decline in a single stock at the management unit scale. The simulation model provides some insight into the collapsed stock – failed recovery dilemma.

Discussion and conclusions

Species that have characteristic social behavior such as group mating, group defense, and schooling may be at increased risk of extinction under heavy exploitation due to Allee effects. Because many commercially important fish species possess such characteristics, failing to recognize and account for Allee effects in fish population dynamics creates a significant management risk. Fisheries management (including stock assessment) must assume the existence of substock structure and devise means for its protection and evaluation. If the conclusions we draw from our modeling are correct, biological reference points developed from aggregated data from multiple substocks and used in conventional fisheries management and stock assessment models are likely to be inaccurate and possibly nonconservative. We recommend that a null recruitment model with a non-zero intercept be examined routinely in stock assessments, even for coarse stock structure analysis.

Recent examination of heavily exploited marine fish populations provides evidence for the existence of Allee effects. For example, Cury et al. (2000) provided an empirical demonstration of the tendency for members of two or more pelagic schooling species of similar size and body shape to form mixed schools when abundance of one of the interacting species was low. The unfortunate outcome of this arrangement may be that the less abundant species would effectively be entrained into the life history behavior of the more abundant species. This would put the species at risk with a critical disadvantage in growth, survival and spawning success — the so-called "school trap." Maintenance of a minimum density critical to the well-being of a population may also apply to other species. Hutchings (1996) has argued that high density, offshore aggregations of Northern cod remained relatively constant over time despite high fishing pressure as a consequence of an immigration of cod from adjacent, less dense areas. This was predicated on the assumption that fitness advantages accrue to being part of a high-density aggregation and that maintenance of such a density is the preferred behavioral strategy. Marshall et al. (1998) have shown that decreased reproductive output associated with adult Northeast Arctic cod in poor physiological condition creates a critical population biomass threshold below which no recruits are produced. Finally, the learning component of fish migratory behavior is believed to be particularly important in those species comprised of multiple age groups, providing the opportunity for social transmission of migration routes (Dodson 1988). This has led investigators to conclude that the altered migration patterns of collapsed cod and herring stocks may have changed irrevocably.

The extent and time scale of recovery of collapsed stocks may be dependent on recolonization from adjacent substocks. Under the assumption of Allee effects, when a substock falls below its critical level, any self-regenerating capabilities have been lost and stock rebuilding can only occur through immigration. Dispersal of offspring or adults from adjacent substocks or populations may fulfill this recolonization role. Research into biophysical modeling and metapopulation theory may be very important in estimating recovery rates. More research in support of delineation of substock structure is also advised.

Finally, our research indicates an immediate need to protect subcomponents of populations from extinction. By the time the status of a species is in question based on conventional fisheries data, population extinction may be well advanced, leading to even greater instability and the probability of irreversible collapse. Indeed, this may be one of the most pressing biodiversity issues facing marine fishes.

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References

Beverton, R.J.H. 1990. Small marine pelagic fish and the threat of fishing: are they endangered? J. Fish. Biol. **37**(Suppl. A): 5–16.

Cury, P., Bakun, A., Crawford, R.J.M, Quinones, R.A., Shannon, L.J., Teichmann, A.J., Verheye, H.M., and Arancibia, H. 2000. Small pelagics in upwelling systems: patterns of interaction and structural changes in "wasp-waist" ecosystems. ICES J. Mar. Sci. In press.

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deYoung, B., and Rose, G.A. 1993. On recruitment and distribution of Atlantic cod (*Gadus morhua*) off Newfoundland. Can. J. Fish. Aquat. Sci. **50**: 2729–2741.

Dodson, J.J. 1988. The nature and role of learning in the orientation and migratory behavior of fishes. Environ. Biol. Fish. **23**: 161–182.

Hutchings, J.A. 1996. Spatial and temporal variation in the density of northern cod and a review of hypotheses for the stock's collapse. Can. J. Fish. Aquat. Sci. **53**: 943–962.

Marshall, C.T., Kjesbu, O.S., Yaragina, N.A., Solemdal, P., and Ulltang, O. 1998. Is spawner biomass a sensitive measure of the reproductive and recruitment potential of Northeast Arctic cod? Can. J. Fish. Aquat. Sci. **55**: 1766–1788.

Myers, R.A., Barrowman, N.J., Hutchings, J.A., and Rosenberg, A.A. 1995. Population dynamics of exploited fish stocks at low population levels. Science (Washington, D.C.), **269**: 1106–1108.

Myers, R.A., Hutchings, J.A., and Barrowman, N.J. 1997*a*. Why do fish stocks collapse? The example of cod in Atlantic Canada. Ecol. Appl. **7**: 91–106.

Myers, R.A., Mertz, G., and Fowlow, P.S. 1997*b*. Maximum population growth rates and recovery times for Atlantic cod, *Gadus morhua*. Fish. Bull. (Washington, D.C.), **95**: 762–772.

Ricker, W.E. 1975. Computation and interpretation of biological statistics of fish populations. Fish. Res. Board Can. Bull. 191.

Shelton, P.A., and Healey, B.P. 1999. Should depensation be dismissed as a possible explanation for the lack of recovery of northern cod (*Gadus morhua*) stock? Can. J. Fish. Aquat. Sci. **56**: 1521–1524.

Sinclair, M., O'Boyle, R., Burke, D.L., and Peacock, G. 1996. Why do some fisheries survive and others collapse? *In* Developing and sustaining world fisheries resources: the state of science and management. 2nd World Fisheries Congress. *Edited by* D.A. Hancock, D.C. Smith, A. Grant, and J.P. Beumer. CSIRO, Australia. pp. 23–35.

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Egg production rates of *Calanus finmarchicus* in the western North Atlantic: effect of gonad maturity, female size, chlorophyll concentration, and temperature

Robert W. Campbell and Erica J.H. Head

Abstract: Egg production rates of *Calanus finmarchicus* were measured in the Labrador Sea (spring) and on the Scotian Shelf (spring, summer, and autumn), in conjunction with indices of gonad maturity and food availability, female body size, surface layer temperature, and surface nitrate concentrations. Egg production rates were often two to four times higher than any previously reported at similar temperatures. Estimates of egg production rates of populations were most closely related to the proportion of reproductively mature females (as measured by a gonad stage index) and female size. Specifically, the gonad stage index (proportion of females capable of spawning) was correlated with the proportion of females that spawned during 24-h incubations, and female size determined the clutch size (i.e., number of eggs produced). Although there were detectable effects of food and temperature on egg production rates, they were generally weaker than the effect of female gonad maturity. Thus, over the broad spatial and temporal scales of this study, the reproductive (i.e., life history) status of a population was more important in determining its egg production rate than direct external factors (i.e., temperature and food conditions).

Résumé : On a mesuré les taux de production d'oeufs de *Calanus finmarchicus* dans la mer du Labrador (printemps) et sur la plate-forme Néo-Écossaise (printemps, été et automne), ainsi que des indices de maturité des gonades et de disponibilité de la nourriture, la taille corporelle des femelles, la température de la couche de surface et les concentrations des nitrates à la surface. Les taux de production d'oeufs étaient souvent de 2 à 4 fois supérieurs à tous ceux déjà signalés à des températures similaires. Les estimations des taux de production d'oeufs dans les populations étaient le plus étroitement corrélées avec la proportion de femelles sexuellement matures (établie à l'aide d'un indice de développement des gonades) et avec la taille des femelles. Plus précisément, l'indice de développement des gonades (indiquant la proportion de femelles capables de pondre) était corrélé avec la proportion de femelles qui ont pondu durant des incubations de 24 h, et la taille des femelles déterminait la taille de la ponte (nombre d'oeufs produits). La disponibilité de la nourriture et la température avaient des effets détectables sur les taux de production d'oeufs, mais ces effets étaient généralement moins importants que l'effet de la maturité des gonades des femelles. Ainsi, dans l'ensemble de l'aire et de la période couvertes par cette étude, le taux de production d'oeufs était davantage fonction du développement des individus en rapport avec la reproduction (cycle vital) dans les populations que de facteurs externes directs comme la température et les conditions alimentaires.

[Traduit par la Rédaction]

Introduction

The copepod *Calanus finmarchicus* is common throughout the North Atlantic (Grainger 1961) and typically represents the greatest biomass of copepods in spring (e.g., McLaren et al. 1989; Gislason and Astthorsson 1995). The eggs and nauplii of copepods are important prey items for the larval stages of many fish species (e.g., Kane 1984), and the abundance and availability of prey at crucial times may be linked to the recruitment of young fish (Cushing 1975). To study the rate of production of these important prey items at critical times, or model the population dynamics of *C. finmarchicus* overall, it is important to quantify the relationships between fecundity and both environmental and biological factors. In addition, copepod egg production rates have been used by a number of authors as indices of feeding and secondary production (e.g., Runge 1985*b*; Berggreen et al. 1988; Peterson et al. 1991).

Egg production rates are easily measured at sea using short-term (<24 h) incubations, which provide realistic estimates of the in situ rate of egg production, independent of short-term laboratory conditions (Hirche 1990; Laabir et al. 1995; Cabal et al. 1997). Previous laboratory and field stud-

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ies have identified endogenous and environmental features that affect egg production rate, including temperature, food availability, female size, and the reproductive maturity of the female population (e.g., Checkley 1980; Runge 1985*a*, 1987; Runge and Plourde 1996).

Spawning by *C. finmarchicus* is a discrete event, whereby a clutch of mature eggs is released directly into the water column (Marshall and Orr 1955). Thus, egg production rate (EPR) can be described as a function of the clutch size (CS) and the time between spawning events (spawning interval (SI)):

$$(1) \qquad \mathrm{EPR}(\mathrm{eggs}\cdot\mathrm{female}^{-1}\cdot\mathrm{day}^{-1}) = \frac{\mathrm{CS}(\mathrm{eggs}\cdot\mathrm{female}^{-1})}{\mathrm{SI}(\mathrm{days})}.$$

Clutch size and the spawning interval are affected by numerous environmental and endogenous factors. Clutch size appears to be partially determined by body size (e.g., prosome length: Corkett and McLaren 1969; Runge and Plourde 1996) and food availability (Corkett and McLaren 1969; Hirche 1990). Spawning interval is sensitive to several factors, including temperature and food availability (Corkett and McLaren 1969; Hirche et al. 1997).

The aim of this study was to examine the effects of variations in environmental conditions (e.g., temperature and food availability) and internal factors (e.g., maternal size and maturity) on in situ rates of egg production of *C. finmarchicus*. To accomplish this, measurements of egg production rate were made during spring, summer, and autumn on the Scotian Shelf and in spring in the Labrador Sea over spatial scales of tens to hundreds of kilometres. Previous sampling (unpublished data) by the Biological Oceanography Section (Ocean Sciences Division) at the Bedford Institute of Oceanography had suggested that this variety of seasons and locales would allow measurements of egg production rates to be made under a variety of different conditions: low temperature and high food concentrations (spring, central Labrador Sea), low temperature and low food concentrations (spring, Labrador and Greenland shelves), high temperature and high food concentrations (autumn, Scotian Shelf, during the autumn bloom), and high temperatures and low food concentrations (summer, Scotian Shelf). In examining the effect of body size, various indices (e.g., prosome length, dry weight, and carbon and nitrogen contents) were used, since they measure different aspects of female size. As well, a mature gonad is a prerequisite for a given female to spawn in an incubation: females that do not possess mature oocytes will not spawn, regardless of temperature and food conditions. Thus, in order to interpret observations of egg production rates, it was important to examine the reproductive status of the population. In this study, a gonad stage index (Niehoff and Hirche 1996) was used to assess the state of reproductive maturity of female populations at all stations where egg production rates were measured.

Materials and methods

Experiments were carried out during three cruises on the Scotian Shelf, in summer (June 5–21) and autumn (November 15–29) of 1996 and in spring (April 18–29) of 1997 and during a cruise to the Labrador Sea also in the spring (May 9 – June 10) of 1997 (Fig. 1). At each station, a suite of hydrographic and biological measurements were made.

Hydrographic and biological sampling

Temperature and in situ fluorescence profiles were taken with a SeaBird® CTD (model 9/11plus) and Chelsea® fluorometer (model Aquatracka III). Water samples for the measurement of chlorophyll *a* were collected using a biological pump or CTD rosette at 10-m intervals from 50 m to the surface.

Zooplankton were collected with a 0.75-m-diameter plankton net (200-μm mesh) towed vertically at ~0.5 $m \cdot s^{-1}$, and time of collection varied among stations. Samples for enumeration, including *C. finmarchicus* females used for staging of gonad maturation, were collected from 100 m (or bottom if <100 m) to the surface and preserved in 2% borax-buffered formalin. The codend was affixed to the hydrowire, so the net sampled only during ascent. Animals for egg production measurements were collected between 50 m and the surface, and the codend was not fixed, allowing the net to collect animals during both ascent and descent. This depth range was used because females reportedly spawn in the near-surface waters (Marshall and Orr 1955), although it is possible that they were sometimes exhibiting diel vertical migration (e.g., Durbin et al. 1995). A 3-L closed codend was used, the contents of which were gently passed through a 0.2-m-diameter, 780-μm sieve submerged in a 10-L pail (to remove smaller copepods and copepodids) and then backwashed into another 10-L pail. Transfers were done as quickly as possible and the bucket was shielded from the light to minimize stress to the animals.

Chlorophyll *a* concentration was used as an index of phytoplankton concentration. Chlorophyll concentrations were determined in 90% acetone extracts of 0.2-μm-filtered water (Whatman GF/F filter) with a Turner Designs® fluorometer (model 10-AU) and the method of Holm-Hansen et al. (1965). Unfortunately, these measurements were not made at all stations. At stations where extracted chlorophyll was not measured, chlorophyll was estimated using the submersible fluorometer mounted on the CTD and by linear regression calibration between extracted chlorophyll and fluorescence (voltage) from the nearest station (usually within 20 km) at which both measurements had been made. There was generally a close correlation ($r^2 = 0.7–0.9$, $n \approx 10$, $p < 0.05$) between chlorophyll and fluorescence profiles at single stations, and examination of the resulting integrated chlorophyll concentrations showed that using these interstation calibrations did not appear to bias the result towards systematically high or low values. Integrated chlorophyll concentrations were calculated from the profiles using the trapezoid rule (Britton 1956) from the surface to 50 m.

Experimental and analytical procedures

Egg production rates were determined using the method of Runge (1985*a*) at a total of 98 stations on the Scotian Shelf (autumn: $n = 8$, summer: $n = 13$, spring: $n = 22$) and in the Labrador Sea ($n = 55$) (Fig. 1). Twenty (1996) or 30 (1997) females were gently pipetted from the freshly collected samples (see above) and placed individually into petri dishes containing 50 mL of 0.2-μm-filtered seawater. The batches of females were then incubated for 24 h in small refrigerators in darkness at temperatures within 2°C of those of the surface waters from which they had been collected. *Calanus finmarchicus* has been observed to have a diel signal in spawning (Runge and Plourde 1996), so the incubation period of 24 h was chosen to encompass a full diel cycle.

Occasionally, *C. glacialis* females were picked by mistake, or females were lost from the dish, reducing the sample size slightly (<10%). It was assumed that cannibalism of the eggs by the females was negligible, as suggested by Laabir et al. (1995) and Cabal et al. (1997). It is uncertain, however, whether the experimental handling of females caused them to spawn prematurely: since there

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is no evidence that this occurs, it was assumed that experimental handling effects were minimal.

Following the 24-h incubation period, the number of eggs produced by each female was counted. The prosome length of each female was measured with an ocular micrometer, and each was then placed in a preweighed 5 × 8 mm tin capsule, dried in a 60°C oven, and stored in a desiccator. The dried females were weighed on a Cahn® model C31 electrobalance and their carbon and nitrogen contents were determined using a Perkin Elmer® model 2400CHN elemental analyzer. Egg production rates for the groups of females were calculated by dividing the number of eggs spawned (during the 24-h incubation) by the total number of females in each incubation.

Gonad maturity was measured in *C. finmarchicus* females from the 0–100 m preserved tows for each of the stations at which egg production rates were measured. Approximately 80 females were stained in 2%:70% borax carmine : ethanol for 24 h and then submerged in 1%:50% HCl:ethanol for 2 h (to remove excess colour from the tissues), dehydrated in an ethanol series (50, 70, 80, 90, and 95%; 2 h at each concentration), and cleared in glycerine for 24–72 h, according to the method of Niehoff and Hirche (1996). The procedure resulted in light pink stained immature oocytes and dark red stained mature oocytes, which were visible through the carapace (which had cleared to near transparency following immersion in the glycerine). Each female was viewed under a dissecting microscope at 50× magnification and assigned to one of the four gonad stage categories as described in Niehoff and Hirche (1996). The gonad stage is expressed as the percentage of females in GS4 (%GS4 hereafter), which is equivalent to the percentage of females in an area capable of spawning.

Statistical methods and analyses

When fitting a straight line ($y = mx + b$) by least squares regression, it is assumed that x is measured without error. If this assumption is not valid, a model II regression is required (Ricker 1973). Model II regressions were calculated using the reduced major axis (RMA) technique, wherever there was uncertainty in the x measurement (i.e., if there was demonstrated variance), even if there was no measure of the variability in x (single values). In order to facilitate comparisons with published work, univariate regressions were performed on egg production rate, clutch size, and proportion spawning to determine the effect of each of the environmental and maternal variables under study. Following that, the variables were combined in multiple linear regressions (fit by ordinary least squares regression). There were several instances where the data were obviously not linear, and those data were $\log_{10}$ transformed.

Egg production rate also equals the mean clutch size divided by the spawning interval (see eq. 1). Since spawning interval can be estimated as the inverse of the frequency of spawning (i.e., the proportion of females that spawned in the 24-h incubations, which is in units of per day) and both terms in eq. 1 have associated uncertainties (assuming a normal distribution for clutch size and a binomial distribution for the proportion spawning), a measure of uncertainty in egg production rate may be estimated. Propagation of errors (presented here as SEs) was performed using the equations of Bevington (1969).

Calanus finmarchicus can release more than one clutch in a 24-h period (Hirche 1996*a*; Hirche et al. 1997), so the assumption that the number of eggs left in the petri dishes equals clutch size may not always be strictly true. Frequency histograms of clutch size at each station were examined and extreme outliers (more than twice the median) were assumed to be double clutches and halved. The probability of obtaining such values by chance alone was <5% for all values (z test). Mean clutch sizes were calculated with the double clutches removed, but double clutches were included in the calculation of egg production rates to avoid biasing egg production rates downward. A total of 18 individuals (out of a total of 2949 females that were observed to spawn) were treated in this manner.

The presence of spatial autocorrelation in each cruise was tested for with Mantel tests (Legendre and Fortin 1989). The Mantel test is based on a cross-product statistic (r_m) that compares the degree of correspondence between matrices (in a manner similar to that of a Pearson correlation) and compares that statistic with a null distribution created by multiple random permutation of one of the matrices. Here, Mantel tests were done on distance matrices of Euclidian distances between all stations for parameters (i.e., $X_{ij} = (\sqrt{X_i X_j})^2$) and spherical distances between stations (calculated from latitude and longitude). The spatial dependence of autocorrelation was examined by assigning stations to one of several distance classes and computing r_m and its associated probability for each distance class. In each test, probabilities were calculated from 1000 random permutations. Tests were not combined among cruises because of the different study areas (Scotian Shelf versus Labrador Sea) and because of potential temporal differences in the populations under study. Given that only four time periods were under study, it was not possible to test for temporal autocorrelation.

Results

Spatial structure

Mantel tests showed the presence of significant spatial correspondence in all variables tested (Table 1, last column). Analysis of the scale dependence of the autocorrelation, however, showed that the variables were generally not autocorrelated on scales <100 km (Table 1). Further analysis showed that the variables were generally significantly autocorrelated on ranges of 100–500 km over the Scotian Shelf and <600 km in the Labrador Sea. Since the scale of the observations (i.e., the distance between two sequential stations) was generally 20–40 km (see Fig. 1), it was assumed that individual stations can be considered independent units for the purpose of statistical tests.

In situ temperature and chlorophyll *a* concentrations

Surface layer temperatures were calculated as the mean temperature between 5 and 20 m. Five metres was chosen as a cutoff point because there were often large variations in the surface temperature. In general, stations of the summer and autumn cruises on the Scotian Shelf showed thermal stratification, while stations of the spring cruises did not. A large range in surface temperatures was observed during the four cruises, from about −1 to 11°C. The coldest temperatures occurred in the Cabot Strait (between Nova Scotia and Newfoundland) and on the Labrador Shelf during spring and the warmest in the waters offshore to the south of the Scotian Shelf during summer.

There was also a large range of integrated chlorophyll concentrations, with both the lowest (12 mg·m^{-2}) and the highest (655 mg·m^{-2}) occurring in the Labrador Sea. In general, chlorophyll concentrations in the Labrador Sea were high over or near the shelves and low in the central basin. Over the Scotian Shelf, chlorophyll concentrations were typically higher offshore and near the shelf break, and they were highest in spring, with summer and autumn exhibiting similar (and lower) concentrations.

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Table 1. Probabilities from Mantel's r_m matrix correspondence test, calculated from 1000 random permutations.

		Distance (km)									
		0–10	10–20	20–30	30–40	40–50	50–60	60–70	70–80	80–90	90–100	All distances
EPR	SS, autumn	—	0.938	0.336	**0.001**	0.69	—	0.251	0.06	0.384	—	**0.027**
	SS, summer	—	0.085	0.885	0.957	0.797	0.663	—	0.701	0.799	0.191	**0.000**
	SS, spring	0.225	0.216	0.526	0.876	0.736	0.919	0.724	0.965	0.849	0.555	**0.006**
	LS, spring	0.102	0.812	0.949	0.891	0.521	0.919	0.794	0.726	0.633	0.645	**0.000**
Nitrogen	SS, autumn	—	0.088	0.292	0.707	0.677	—	0.66	0.745	**0.005**	—	**0.003**
	SS, summer	—	0.988	0.868	0.684	0.962	0.84	—	0.8	0.917	**0.012**	**0.000**
	SS, spring	0.677	0.038	0.967	0.545	0.8	0.469	0.649	0.844	0.755	0.115	**0.000**
	LS, spring	0.923	0.716	0.774	0.942	0.364	0.696	0.865	0.046	0.401	0.677	**0.000**
%GS4	SS, autumn	—	0.601	0.692	**0.014**	0.352	—	0.35	0.163	0.777	—	**0.001**
	SS, summer	—	0.417	0.647	0.006	0.636	**0.022**	—	0.257	0.196	0.135	**0.008**
	SS, spring	0.344	0.707	0.41	0.985	0.573	0.991	0.247	0.983	0.107	0.622	**0.000**
	LS, spring	0.419	0.901	0.961	0.729	0.967	0.372	0.56	0.636	0.446	0.964	**0.000**
Temperature	SS, autumn	—	0.531	0.851	0.889	0.74	—	0.697	0.894	0.877	—	**0.000**
	SS, summer	—	0.561	0.147	0.289	0.93	0.406	—	0.053	0.837	0.502	**0.000**
	SS, spring	0.077	0.689	0.962	0.776	1	0.616	0.862	0.974	0.862	0.962	**0.000**
	LS, spring	0.921	0.911	0.511	0.929	0.158	1	0.444	0.967	0.81	0.374	**0.000**
Chl *a*	SS, autumn	—	0.642	0.403	0.824	0.257	—	0.463	**0.004**	0.899	—	**0.009**
	SS, summer	—	0.778	0.499	0.64	0.918	0.966	—	0.183	0.654	0.181	**0.000**
	SS, spring	0.889	0.997	0.165	0.817	0.6	0.326	0.272	0.166	0.312	0.994	**0.000**
	LS, spring	0.63	0.706	0.827	0.759	0.4	0.978	0.985	0.946	0.912	0.862	**0.000**

Note: SS, Scotian Shelf; LS, Labrador Sea. Tests where the probability of observing a similar pattern (in the two matrices tested) by chance alone was <5% are boldfaced.

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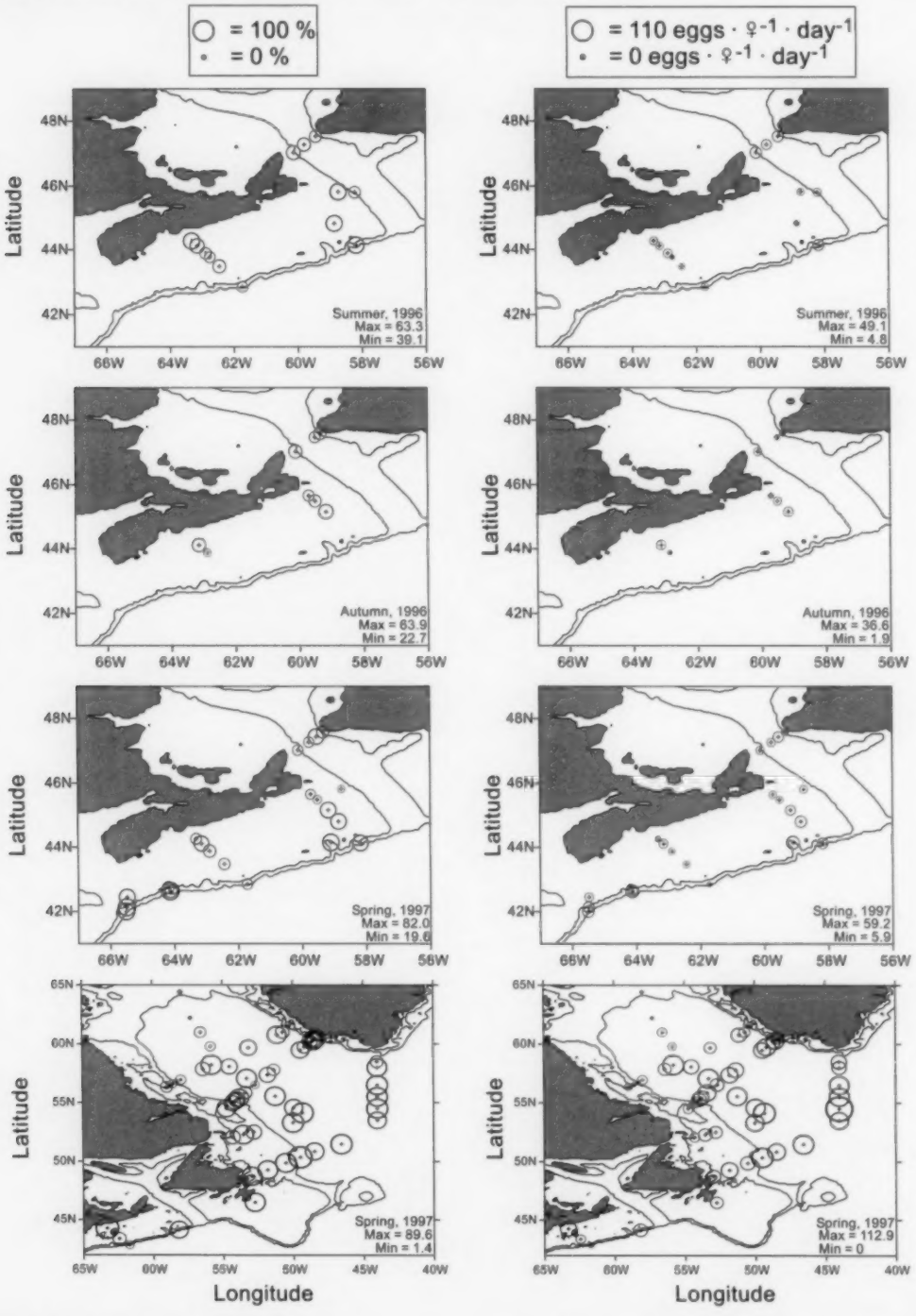
Fig. 1. Proportion of *C. finmarchicus* females with mature gonads (0–100 m, %GS4, left panels) and egg production rate (0–50 m, right panels) on the Scotian Shelf and in the Labrador Sea. The 200- and 1000-m isobaths are shown.



Reproductive status of *C. finmarchicus* females

The proportion of females in the 0–100 m depth range that were in a state considered ready to spawn (i.e., %GS4) varied both spatially and temporally (Fig. 1). In the Labrador Sea in spring, most females were in GS4 at most stations, but there were also several stations where few females were ripe, particularly to the northwest, along the margin of the basin, and on the Labrador Shelf. On the Scotian Shelf, the

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Fig. 2. Relationship between egg production rates for *C. finmarchicus* females (0–50 m) and (*a*) percentage of females in GS4 (0–100 m), (*b*) mean female nitrogen content, (*c*) integrated chlorophyll *a* concentration (0–50 m), and (*d*) mean surface layer temperature (5–20 m). The lines were fit by RMA regression. n = 98; bars = SE. Symbols denote cruises: solid circles, Labrador Sea, May–June 1997; open circles, Scotian Shelf, April–May 1997; squares, Scotian Shelf, June 1996; triangles, Scotian Shelf, November 1996.

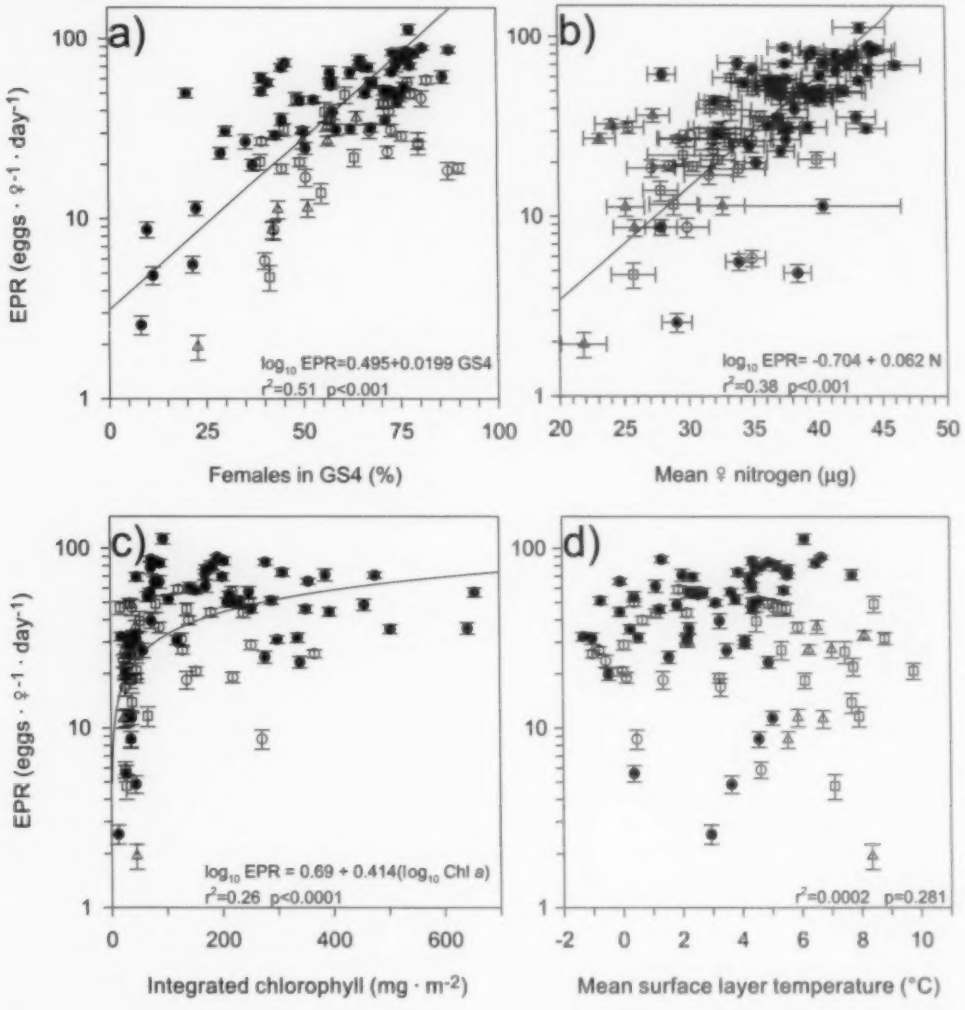

Table 2. Pearson's product-moment correlation coefficient matrix for *C. finmarchicus* body size measures.

	Carbon	Nitrogen	Prosome length
Nitrogen	0.383		
Prosome length	0.595	0.772	
Dry weight	0.809	0.689	0.726

Note: All correlations significantly different from zero with associated probabilities <0.001.

proportion of ripe females was generally highest in spring, lowest in autumn, and intermediate in summer.

Variations in female size

Nitrogen content, prosome length, carbon content, and dry weight varied in slightly different ways between seasons and locales, but in general, all indices of female size were lowest in the autumn (Scotian Shelf) and highest in the spring (Scotian Shelf and (or) Labrador Sea). All measures of body size were positively correlated (Table 2). Although the lipid content of the females was not measured directly, microscopic observations showed that the size of the lipid sac varied considerably in live females.

There was also a significant relationship between clutch size and prosome length (CS = –205.663 + length × 95, r^2 = 0.248, $p < 0.001$; RMA regression: CS = –469.34 + length × 187.74, $p < 0.001$). The relationship between clutch size and prosome length is similar to that reported by Runge and Plourde (1996) (CS = –192.4 + length × 91.8).

Egg production rate, clutch size, and spawning frequency

Egg production rates for groups of females varied between 0 and 113 eggs·female^{-1}·day^{-1}, and both the highest and lowest rates were observed in the Labrador Sea (Fig. 1).

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In the Labrador Sea in spring, egg production rates tended to be higher towards the centre of the basin (i.e., egg production rates were lower on the shelves), and they also tended to be somewhat higher in spring in waters offshore of the Scotian Shelf. It is difficult to draw conclusions about spatial patterns of egg production over the Scotian Shelf in summer or autumn, since the spatial coverage was rather limited (Fig. 1). Variance in the egg production rate, clutch size, and proportion spawning was occasionally considerable (CV of 60–70%), but CV averaged 30% for clutch size, 19% for the proportion spawning, and 38% for egg production rate.

The best single predictor of egg production rate, in terms of the proportion of variance explained, was the proportion of females in GS4 (Fig. 2*a*). Because the gonad stage (%GS4) is a measure of the reproductive status of the population, it should be most closely related to the proportion of females observed to spawn during the 24-h incubations. This was indeed the case, with %GS4 accounting for 66% of the variance in the percentage of females observed to spawn (Fig. 3*a*). There was also a significant positive relationship between %GS4 and the mean clutch size (Fig. 3*b*), but a smaller proportion of variance (14%) was explained by that relationship (summarized in Table 2).

After %GS4, the best predictor of egg production rate was mean female nitrogen content (Fig. 2*b*). Although mean nitrogen content was the "best" measure of body size (in terms of explaining the variance in egg production), there were also significant relationships between $\log_{10}$ egg production rate and mean prosome length ($r^2 = 0.20$, $p < 0.001$) or mean dry weight ($r^2 = 0.11$, $p = 0.001$) but not with mean carbon content ($r^2 = 0.0003$, $p = 0.85$). Mean nitrogen content, which was measured after the incubations, might have been anticipated to vary depending on whether or not an individual female had spawned. However, when all females at each station were grouped according to whether or not they had spawned, there was no significant difference among stations (means ± SDs of 35.58 ± 6.68 and 35.43 ± 6.64 µg, respectively; $n = 98$, two-tailed t test, $p = 0.82$). Neither carbon content nor dry weight was closely correlated with egg production rate. This was not unexpected, since it is likely that egg production rate is a function of the structural mass of the copepod (McLaren and Leonard 1995), whereas carbon content and dry weight are measures of structural mass and lipid combined (see above).

Mean nitrogen content explained 61% of the variance in mean clutch size (Fig. 4*a*), and there was a weaker but still significant relationship between clutch size and mean prosome length ($r^2 = 0.25$, $p < 0.001$). In addition, there was also a weak relationship ($r^2 = 0.14$, $p < 0.001$) between mean nitrogen content and the proportion of females observed to spawn during the 24-h incubation period (Fig. 4*b*).

Although mean clutch size and the proportion spawning varied with both mean nitrogen content and %GS4 (respectively) in the univariate sense, they were combined into a multiple linear regression to examine their combined effects. Nitrogen content and %GS4 are used as independent predictors because they appear to be independent of each other ($r^2 = 0.04$, $p < 0.001$). Similarly, multiple linear regression of %GS4 and mean nitrogen content on the proportion spawning also resulted in a small (3%) increase in the proportion of variance explained over the model including only %GS4 (Table 3). Multiple linear regression of mean nitrogen content and %GS4 on $\log_{10}$-transformed egg production rate, however, resulted in a much higher proportion of variance being explained over the %GS4 only model (19%) (Table 3).

Effect of food availability on egg production rate

Egg production rates were very variable at low integrated chlorophyll concentrations and tended toward a limit at higher levels; the relationship could be described by an asymptotic model (Fig. 2*c*: a log chlorophyll model is used for an asymptotic model). Since egg production rate was not independent of nitrogen content and %GS4, these two factors might have led to some of the scatter about the line. Therefore, the effect of body size on egg production rate was incorporated by using a nitrogen-specific egg production rate (assuming an egg nitrogen content of 0.044 µg; Runge and Plourde 1996); the effect of %GS4 could not be included in the model, since there is no simple way to incorporate it logically. A $\log_{10}$-transformed chlorophyll model of nitrogen-specific egg production rate actually explained less variance ($r^2 = 0.21$, $p < 0.001$) than the model in which egg production rate was not normalized to nitrogen content (Fig. 2*c*). Addition of a $\log_{10}$ chlorophyll term (chlorophyll was $\log_{10}$ transformed to conform to the assumption of linearity) into the multiple linear regression of mean nitrogen content and %GS4 on clutch size, proportion spawning, or $\log_{10}$ egg production rate resulted in a small increase in the proportion of variance explained (<3% in all cases) (Table 3).

It is possible that an asymptotic function is not appropriate for modeling the response of egg production rate to food concentration. For example, egg production might be limited at low food concentrations but not affected at high concentrations (e.g., Checkley 1980). Thus, a bilinear model, with a fitted break point, was fitted to the nitrogen-specific egg production rate by least squares (described by Post and Lee 1996). A two-line model explained more variance in nitrogen-specific egg production rate ($r^2 = 0.33$, $p < 0.001$) than either saturation model, and the break point of the two lines corresponded to a chlorophyll concentration of 79 $mg \cdot m^{-2}$ (Fig. 5*a*, broken vertical line). Below 79 mg Chl $a \cdot m^{-2}$, there was a positive relationship between chlorophyll concentration and nitrogen-specific egg production rate ($r^2 = 0.26$, $p < 0.001$) (Fig. 5*b*). Multiple linear regression of nitrogen-specific egg production rate on chlorophyll and %GS4 at those stations, however, showed that the effect of chlorophyll was secondary to that of gonad maturity, as evidenced by a 48% increase in r^2 with the inclusion of the %GS4 term (nitrogen-specific EPR = 4.7×10^{-4}(Chl a) + 8.08×10^{-4}(%GS4) – 2.12×10^{-2}, $r^2 = 73.8$, $p < 0.001$).

Effect of temperature on egg production rate

The $\log_{10}$ egg production was essentially independent of surface layer temperature (Fig. 2*d*). Substitution of nitrogen-specific egg production rate on the ordinate did not identify a relationship with temperature either ($r^2 = 0.002$, $p = 0.89$). Furthermore, the addition of a temperature term to the multiple linear regressions did not increase the r^2 (in fact, the r^2 was reduced over the %GS4 and nitrogen only model in every instance) (Table 3). As well, the coefficient for tempera-

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Fig. 3. Relationship between the percentage of *C. finmarchicus* females in GS4 (0–100 m) and (*a*) the percentage of females observed to spawn during the 24-h incubation period (0–50 m) and (*b*) mean clutch size. The lines were fit by RMA regression. n = 98; bars = SE. Symbols as in Fig. 2.

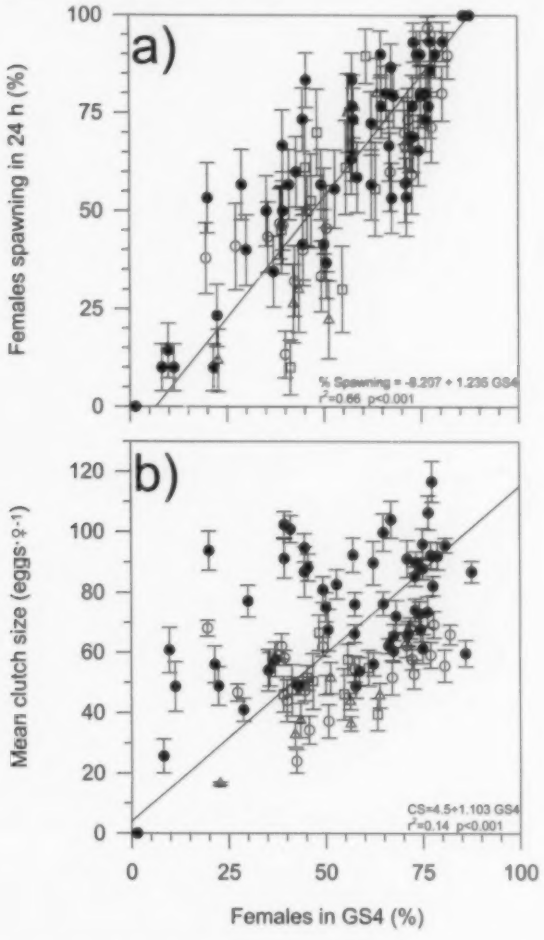
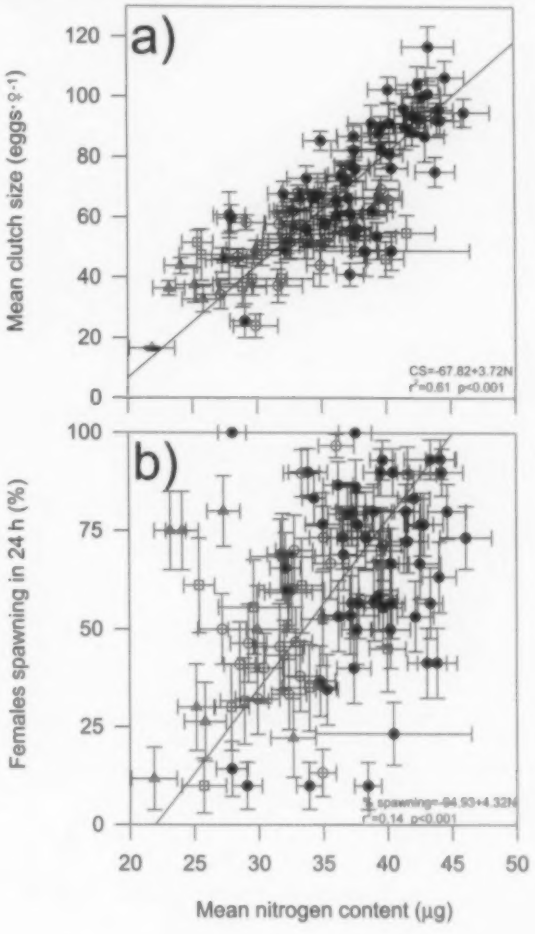


Fig. 4. Relationship between mean nitrogen content and (*a*) clutch size and (*b*) percentage of *C. finmarchicus* females observed to spawn over the 24-h incubation period. The lines were fit by RMA regression. n = 98; bars = SE. Symbols as in Fig. 2.



ture in all of the multiple linear regressions was also not significantly different from zero (Table 3). It is possible that the production of eggs may be limited by food availability at low food concentrations and under the influence of other factors beyond a threshold amount of food availability. At integrated chlorophyll concentrations >79 mg·m^{-2}, where nitrogen-specific egg production rate appeared to be independent of chlorophyll concentration, however, temperature explained only a modest amount of the variance in nitrogen-specific egg production rate (nitrogen-specific EPR = $3.4 \times 10^{-3} + 9.0 \times 10^{-3}$(temperature), $r^2 = 0.14$, $p = 0.003$).

Discussion

Egg production rates of groups of female *C. finmarchicus* from the 0–50 m depth range at stations throughout the Northwest Atlantic (Scotian Shelf and Labrador Sea) were closely related to the state of reproductive maturity of the female population within the 0–100 m depth range. These results are not surprising, since %GS4 is defined as the proportion of females capable of spawning. Moreover, they are consistent with the observations of Runge (1987), who found that 75% of the variance in egg production (near Browns Bank, Scotian Shelf) was attributable to the proportion of females in his gonad stages 4–7 (roughly equivalent to %GS4). Gonad maturity affected egg production rates by its effect on the proportion of females that spawned in the 24-h incubations, which is consistent with the observations of Niehoff and Hirche (1996), who observed a close relationship between %GS4 and the proportion of females spawning in their incubations. A second, less important effect was a tendency toward larger clutch sizes in areas where there was a higher proportion of mature females (Fig. 3*b*). Overall, however, %GS4 did not seem to be very important in determining clutch size because its inclusion (along with mean nitrogen content) into the multiple linear regression of clutch size did not appreciably increase the proportion of variance explained (Table 3).

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Table 3. Summary of regression models for clutch size, proportion of *C. finmarchicus* females spawning, and $\log_{10}$-transformed egg production rate regressed on mean nitrogen content, proportion of females in GS4, integrated chlorophyll concentration (0–50 m), and surface layer temperature (n = 98 for all regressions).

	Clutch size		% spawning		$\log_{10}$ egg production rate	
	Coefficient	r^2	Coefficient	r^2	Coefficient	r^2
Constant	–67.82 ($p < 0.001$)	0.61 ($p < 0.001$)	–8.21 ($p = 0.93$)	0.66 ($p < 0.001$)	0.79 ($p = 0.43$)	0.51 ($p < 0.001$)
Nitrogen	3.72 ($p < 0.001$)		1.24 ($p < 0.001$)		0.01 ($p < 0.001$)	
Constant	4.50 ($p < 0.001$)	0.14 ($p < 0.001$)	–1.24 ($p = 0.32$)	0.14 ($p < 0.001$)	0.15 ($p < 0.001$)	0.38 ($p < 0.001$)
%GS4	1.10 ($p < 0.001$)		1.69 ($p < 0.001$)		0.04 ($p < 0.001$)	
Constant	–42.74 ($p < 0.001$)	0.64 ($p < 0.001$)	–22.57 ($p = 0.02$)	0.69 ($p < 0.001$)	–0.11 ($p = 0.41$)	0.70 ($p < 0.001$)
Nitrogen	2.68 ($p < 0.001$)		0.95 ($p = 0.001$)		0.03 ($p < 0.001$)	
%GS4	0.23 ($p = 0.002$)		0.85 ($p < 0.001$)		0.01 ($p < 0.001$)	
Constant	–48.94 ($p < 0.001$)	0.65 ($p < 0.001$)	–29.10 ($p = 0.003$)	0.71 ($p < 0.001$)	–0.24 ($p = 0.07$)	0.73 ($p < 0.001$)
Nitrogen	2.50 ($p < 0.001$)		0.65 ($p = 0.001$)		0.02 ($p < 0.001$)	
%GS4	0.19 ($p = 0.01$)		0.90 ($p < 0.001$)		0.01 ($p < 0.001$)	
$\log_{10}$ Chl *a*	7.36 ($p = 0.03$)		7.69 ($p = 0.03$)		0.16 ($p = 0.002$)	
Constant	–41.28 ($p < 0.001$)	0.63 ($p < 0.001$)	–25.69 ($p = 0.007$)	0.70 ($p < 0.001$)	–0.09 ($p = 0.50$)	0.70 ($p < 0.001$)
Nitrogen	2.68 ($p < 0.001$)		0.86 ($p = 0.001$)		0.03 ($p < 0.001$)	
%GS4	0.22 ($p = 0.003$)		0.95 ($p < 0.001$)		0.01 ($p < 0.001$)	
Temperature	–0.31 ($p = 0.50$)		0.67 ($p = 0.17$)		–0.003 ($p = 0.63$)	

Note: Simple linear regressions were fit by RMA regression; multiple linear regressions were fit by least squares. Probability values associated with coefficients test the null hypothesis that the coefficient equals zero (t test, two tails) and probability values associated with r^2 are from regression ANOVAs.

If %GS4 is a measure of females capable of spawning, then plots of the proportion spawning versus %GS4 should have fallen on the 1:1 line (e.g., if the %GS4 was 75%, then 75% of the females should have spawned in the 24-h incubation). If females required longer than 24 h to produce a clutch, then observations could have fallen below the 1:1 line (e.g., if %GS4 was 75% but only 50% spawned over the 24-h period). At no time should a point have occurred above the 1:1 line, since this would indicate that more females were spawning than were measured as actually capable of doing so (e.g., a situation where %GS4 was 75% but 100% of females spawned). In Fig. 3*a*, 50% of the observations were above the 1:1 line, many by ~25%, and the model II regression line actually crossed the 1:1 line. This may have been due in part to uncertainty in the %GS4 measurement and the subjective nature of the measurement. In addition, it is likely that %GS4 in the populations was often <100% because the females used for those assessments were collected from 0–100 m, while the experimental animals were collected from 0–50 m. Females in the 0–50 m depth range may actually have been more mature, since *C. finmarchicus* overwinters at depth and presumably does not enter the near-surface waters until ready to spawn (e.g., Hopkins et al. 1984). Groups of females collected in the deeper tows may thus have contained a higher proportion of immature individuals, biasing the measurement of %GS4 toward lower values and moving the entire relationship in the negative direction along the abscissa. The importance of this effect cannot be assessed because it depends on the difference in gonad stage between the animals in the 0–100 m preserved tows and the animals used for the egg production measurements in the 0–50 m tows, which is unknown for any one station and which may have varied among stations.

The factors that mediate the state of gonad maturity in nature are unclear. The developmental status of the population is certainly important, since *C. finmarchicus* tends to overwinter in an immature state (Hirche 1996*b*). Food availability is also likely important in the maturation process (Plourde and Runge 1993; Niehoff and Hirche 1996). Plourde and Runge (1993) observed an about 10-day lag between the onset of the spring phytoplankton bloom and maximal egg production rates, which they attributed to the time required for the maturation of the gonad. The factors mediating emergence from diapause are also unclear; they may be environmental (e.g., light intensity, day length, temperature: Hirche 1996*b*; Miller et al. 1991) or endogenous (i.e., an "internal clock": Miller et al. 1991). *Calanus finmarchicus* probably undergoes primary vitellogenesis while in diapause (in stage CV) and completes secondary vitellogenesis following emergence from diapause (Niehoff and Hirche 1996). This secondary phase may thus be sensitive to environmental factors such as temperature and food availability. In this study, however, the proportion of reproductively mature females (%GS4) was not obviously related to any environmental variables or endogenous features of the females.

Egg production rate was influenced by female body size via its effect on clutch size. Examination of the results from the multiple linear regressions shows that clutch size was independent of temperature or food concentration. Multiple linear regression of both nitrogen content and gonad stage on $\log_{10}$ egg production rates showed that body size (as measured by nitrogen content) was secondary to gonad stage. Addition of the female size term, however, did increase the proportion of variance explained by 19%, suggesting that it is not an insignificant factor. It is perhaps not surprising to observe that egg production rate is related to female size, given that many metabolic rates scale allometrically.

Egg production rates of female *C. finmarchicus* were only

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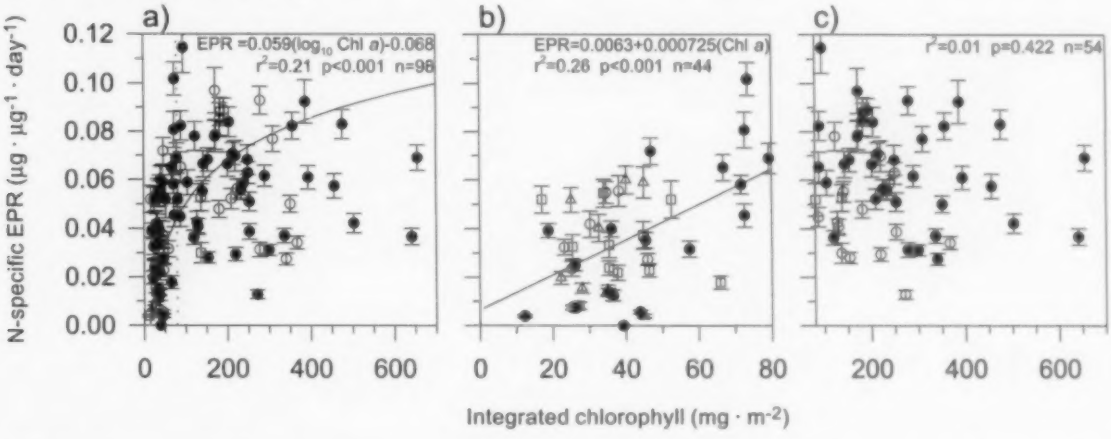
Fig. 5. (*a*) Relationship between *C. finmarchicus* female nitrogen-specific egg production rate and integrated chlorophyll *a* concentration (0–50 m). The broken vertical line indicates the break point (79 mg·m^{-2}) from a bilinear model. Bars = SE. (*b* and *c*) Relationship between nitrogen-specific egg production rate and integrated chlorophyll *a* concentration (0–50 m) at stations where integrated chlorophyll was <79 mg·m^{-2} (Fig. 5*b*) and at stations where integrated chlorophyll was >79 mg·m^{-2} (Fig. 5*c*). The lines were fit by RMA regression. Bars = SE. Symbols as in Fig. 2.



weakly related to in situ food concentration (as estimated by integrated chlorophyll concentration). The conversion of egg production rates (eggs per female per day) to nitrogen-specific rates (micrograms of egg nitrogen per microgram of female nitrogen per day) did not improve upon the proportion of variance explained by chlorophyll concentration for $\log_{10}$-transformed egg production rate. Moreover, the small effect of $\log_{10}$ chlorophyll in the multiple linear regressions suggests that the effect of food concentration is tertiary to that of gonad maturity and female size.

Assignment of integrated chlorophyll into two categories, "high" and "low" (above and below 79 mg·m^{-2}), with the bilinear model suggested that nitrogen-specific egg production rate was independent of chlorophyll concentration at high concentrations. At low chlorophyll concentrations, however, there was a weak (but significant) relationship with nitrogen-specific egg production rate, which was secondary to the overriding influence of gonad stage.

According to reports in many previous laboratory and field studies, egg production rate increases with increasing temperature (e.g., Plourde and Runge 1993; Runge 1985*b*; Hirche et al. 1997). The data presented here, however, did not show such a relationship. Conversion of egg production rate into units used by previous workers (Fig. 6) showed a great deal of scatter about the empirical and theoretical models (Runge and Plourde (1996) and McLaren and Leonard (1995), respectively), and again, no obvious relationship could be discerned between our estimates of egg production rate and temperature (Fig. 2*d*). At nonlimiting food concentrations (integrated chlorophyll concentration >79 mg·m^{-2}), there was a weak relationship between nitrogen-specific egg production rate and surface layer temperature, but the proportion of ripe females was much more important in determining the observed egg production rates. It might be suggested that the temperature in the incubations may have influenced the observed egg production rates if they were different from in situ temperatures. Egg production rates estimated in 24-h incubations, however, are apparently quite robust with respect to experimental conditions of both food and temperature (see Laabir et al. 1995). Moreover, the incubation temperatures here were generally within 1–2°C of the in situ surface layer temperature, although there was uncertainty about the exact temperature that the females were experiencing throughout the day, since their vertical distribution in the top 50 m of the water column was not directly observed. Overall, however, the variance in the weight-specific egg production rates was apparently not due to temperature effects but rather to the effects of the reproductive status of the female population (%GS4) and food availability.

If egg production is a measure of secondary production, then specific egg production rates should be equivalent to specific growth rates. Several of the specific egg production rates reported here were higher than any previously reported for *C. finmarchicus*, particularly those found at low temperatures. Hirche (1990) found carbon-specific egg production rates for *C. finmarchicus* of 5.5% C·day^{-1} at 0°C in the East Greenland Current (Greenland Sea), and Hirche et al. (1997) found rates of 2.1 and 3% C·day^{-1} at –1.5 and 0°C, respectively (in laboratory-maintained populations). At higher temperatures, Hirche et al. (1997) found carbon-specific rates of 7.4 and 8.5% C·day^{-1} at 5 and 8°C, respectively, and Runge and Plourde (1996) gave rates of ~9 and 12% C·day^{-1} at 10 and 13.5°C, respectively. The specific rates found in this study were often much higher (two to four times) than those expected at all temperatures, and several exceeded previously published maximal egg production rates. This suggests that *C. finmarchicus* in the western North Atlantic may grow at rates much higher that previously thought and provides further evidence that the spawning of *C. finmarchicus* is not limited by temperature, as discussed in Hirche (1990).

Differences between the egg production rates reported here and those previously published may have resulted partly from the use of carbon as the unit of measurement for egg production rate and female size. The metabolic substrate for carrying out physiological processes, including the production of eggs, resides in the structural mass of the cope-

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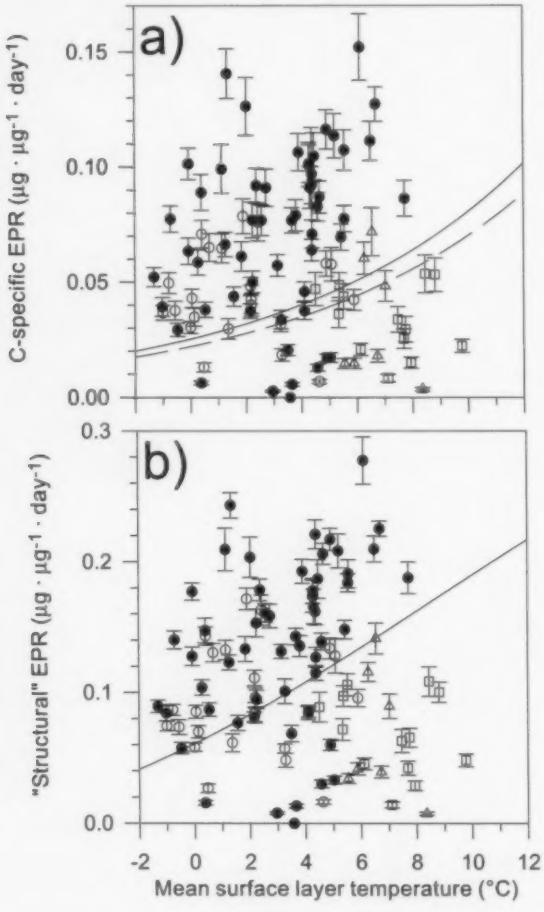
Fig. 6. Relationship between mean surface layer temperature and *C. finmarchicus* female (*a*) carbon-specific egg production rate and (*b*) "structural" specific egg production rate. The lines in Fig. 6*a* are from Runge and Plourde (1996, their fig. 2) based on a dry weight to carbon ratio (including lipids) of 0.54 (EPR = 2.198 $e^{0.116T}$, broken line) or 0.47 (EPR = 2.525 $e^{0.116T}$, solid line). The line in Fig. 6*b* is from McLaren and Leonard (1995, their fig. 3) representing temperature-corrected growth rates of copepodid stages (EPR = $\exp[0.114 - 0.00318(T - 5)][279(T + 10.6)^{-2.05}]^{-1} - 1$). Structural egg production rates were derived from the regression for *C. finmarchicus* of McLaren and Leonard (1995): structural weight = $e^{1.919+3.3\ \ln(\text{prosome length})}$, $n = 98$; bars = SE. Symbols as in Fig. 2.



pod (McLaren and Leonard 1995). The carbon content of the entire copepod, however, includes carbon in the form of lipid reserves as well as carbon associated with the structural mass. Lipid reserves are usually incorporated in measures of carbon content, since their removal is difficult and time consuming. Furthermore, enhanced maternal lipid content does not necessarily result in increased fecundity because egg lipids (triacylglycerols) must be synthesized from storage lipids (wax esters) or obtained from phytoplankton (Miller et al. 1998). Thus, carbon-specific rates in lipid-rich animals will tend to underestimate "structural" specific rates. Indeed, the weaker correlations between measures of structural body size (i.e., nitrogen contents and prosome length) and those that include lipid (i.e., carbon contents and dry weight) in Table 2 are likely the result of variable amounts of lipid reserves. In this study, we did not explicitly measure or remove lipids, so many of the carbon-specific rates shown here were also presumably underestimates of structural carbon specific rates, although the higher rates are likely closer to actual structural carbon-specific growth rates. Published carbon-specific rates that include lipid contents should thus be regarded as underestimates; future studies should remove lipid contents (for carbon-specific rates) or use lipid-independent measurements (e.g., nitrogen content).

It is possible that temperature and food relationships were detected in previous studies because they used females that belonged to the same population (e.g., females from a relatively confined area such as an estuary or a fjord or from a population maintained for some time in vitro). These types of "within-population" studies predominate in the egg production literature, particularly for *C. finmarchicus* (e.g., Marshall and Orr 1955; Runge 1985*b*; Hirche 1990; Diel and Tande 1992; Plourde and Runge 1993; Hirche et al. 1997). Within these studies, it seems likely that effects of gonad stage and female size may be "controlled for" in that they would have been similar within populations, so that temperature and food effects would have been more important (and thus more easily detected).

The production of eggs is a complex problem for copepods in nature. Reproductive output (i.e., fecundity) must be balanced with numerous factors, including physiological limitations, nutrition (quantity and quality of food), and predation risk. Moreover, prior to the production of any eggs, a female must undergo the gonad maturation process, which is itself affected by the same factors. This study has shown that over large spatial and temporal scales, egg production rates are primarily a function of the state of gonad maturity of the population, secondarily a function of mean female body size, and only weakly related to food and temperature.

Acknowledgments

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References

Berggreen, U., Hansen, B., and Kiørboe, T. 1988. Food size spectra, ingestion and growth of the copepod *Acartia tonsa* during development: implications for determination of copepod production. Mar. Biol. **99**: 341–352.

Bevington, P.R. 1969. Data reduction and error analysis for the physical sciences. McGraw-Hill, New York.

Britton, J.R. 1956. Calculus. Rinehart and Winston, New York.

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Cabal, J., Harris, L.R., and Head, E.J.H. 1997. Egg production rates of *Calanus finmarchicus* in the Northwest Atlantic. Can. J. Fish. Aquat. Sci. **54**: 1270–1279.

Checkley, D.M., Jr. 1980. Food limitation of egg production by a marine, planktonic copepod in the sea off southern California. Limnol. Oceanogr. **25**: 991–998.

Corkett, C.J., and McLaren, I.A. 1969. Egg production and oil storage by the copepod *Pseudocalanus* in the laboratory. J. Exp. Mar. Biol. Ecol. **3**: 90–105.

Cushing, D.H. 1975. Marine ecology and fisheries. Cambridge University Press, Cambridge, U.K.

Diel, S., and Tande, K. 1992. Does the spawning of *Calanus finmarchicus* in high latitudes follow a reproducible pattern? Mar. Biol. **113**: 21–31.

Durbin, E.G., Gilman, S.L., Campbell, R.G., and Durbin, A.G. 1995. Abundance, biomass, vertical migration and estimated development rate of the copepod *Calanus finmarchicus* in the southern Gulf of Maine during late spring. Continental Shelf Res. **15**: 571–591.

Gislason, A., and Astthorsson, O.S. 1995. Seasonal cycle of zooplankton southwest of Iceland. J. Plankton Res. **10**: 151–169.

Grainger, E.H. 1961. The copepods *Calanus glacialis* Jaschnov and *Calanus finmarchicus* (Gunnerus) in Canadian Arctic–Subarctic waters. J. Fish. Res. Board Can. **18**: 663–678.

Hirche, H.J. 1990. Egg production of *Calanus finmarchicus* at low temperature. Mar. Biol. **106**: 53–58.

Hirche, H.J. 1996*a*. The reproductive biology of the marine copepod, *Calanus finmarchicus* — a review. Ophelia, **44**: 111–128.

Hirche, H.J. 1996*b*. Diapause in the marine copepod, *Calanus finmarchicus* — a review. Ophelia, **44**: 129–143.

Hirche, H.J., Meyer, U., and Niehoff, B. 1997. Egg production of *Calanus finmarchicus*: effect of temperature, food and season. Mar. Biol. **127**: 609–620.

Holm-Hansen, O., Jorenzen, C.J., Holmes, R.W., and Strickland, J.D.H. 1965. Fluorimetric determination of chlorophyll. J. Cons. Int. Explor. Mer, **30**: 3–15.

Hopkins, C.C.E., Tande, K.S., Grønvik, S., and Sargent, J.R. 1984. Ecological investigations of the zooplankton community of Balsfjorden, northern Norway: an analysis of growth and overwintering tactics in relation to niche and environment in *Metridia longa* (Lubbock), *Calanus finmarchicus* (Gunnerus), *Thysanoessa inermis* (Krøyer) and *T. Rasschi* (M. Sars). J. Exp. Mar. Biol. Ecol. **82**: 77–99.

Kane, J. 1984. The feeding habits of co-occurring cod and haddock larvae from Georges Bank. Mar. Ecol. Prog. Ser. **16**: 9–20.

Laabir, M., Poulet, S.A., and Ianora, A. 1995. Measuring production and viability of eggs in *Calanus helgolandicus*. J. Plankton Res. **17**: 1125–1142.

Legendre, P., and Fortin, M. 1989. Spatial pattern and ecological analysis. Vegetatio, **80**: 107–138.

Marshall, S.M., and Orr, A.P. 1955. The biology of a marine copepod *Calanus finmarchicus*. Oliver and Boyd, Edinburgh, U.K.

McLaren, I.A., and Leonard, A. 1995. Assessing the equivalence of growth and egg production of copepods. ICES J. Mar. Sci. **52**: 397–408.

McLaren, I.A., Tremblay, J.M., Corkett, C.J., and Roff, J.C. 1989. Copepod production on the Scotian Shelf based on life-history analyses and laboratory rearings. Can. J. Fish. Aquat. Sci. **46**: 560–583.

Miller, C.B., Cowles, T.J., Wiebe, P.H., Copley, N.J., and Grigg, H. 1991. Phenology in *Calanus finmarchicus*; hypotheses about control mechanisms. Mar. Ecol. Prog. Ser. **72**: 79–91.

Miller, C.B., Morgan, C.A., Prahl, F.G., and Sparrow, M.A. 1998. Storage lipids of the copepod *Calanus finmarchicus* from Georges Bank and the Gulf of Maine. Limnol. Oceanogr. **43**: 488–497.

Niehoff, B., and Hirche, H.J. 1996. Oogenesis and gonad maturation in the copepod *Calanus finmarchicus* and the prediction of egg production from preserved samples. Polar Biol. **16**: 601–612.

Peterson, W.T., Tiselius, P., and Kiørboe, T. 1991. Copepod egg production, moulting and growth rates, and secondary production in the Skagerrak in August 1988. J. Plankton Res. **13**: 131–154.

Plourde, S., and Runge, J.A. 1993. Reproduction of the planktonic copepod *Calanus finmarchicus* in the lower St. Lawrence Estuary: relation to the cycle of phytoplankton production and evidence for a *Calanus* pump. Mar. Ecol. Prog. Ser. **102**: 217–227.

Post, J.R., and Lee, J.A. 1996. Metabolic ontogeny of teleost fishes. Can. J. Fish. Aquat. Sci. **53**: 910–923.

Ricker, W.E. 1973. Linear regressions in fishery research. J. Fish. Res. Board Can. **30**: 409–434.

Runge, J.A. 1985*a*. Relationship of egg production of *Calanus pacificus* to seasonal changes in phytoplankton availability in Puget Sound, Washington. Limnol. Oceanogr. **30**: 382–396.

Runge, J.A. 1985*b*. Egg production rates of *Calanus finmarchicus* in the sea off Nova Scotia. Arch. Hydrobiol. Beih. **21**: 33–40.

Runge, J.A. 1987. Measurement of egg production rate of *Calanus finmarchicus* from preserved samples. Can. J. Fish. Aquat. Sci. **44**: 2009–2012.

Runge, J.A., and Plourde, S. 1996. Fecundity characteristics of *Calanus finmarchicus* in coastal waters of eastern Canada. Ophelia, **44**: 171–187.

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Cadmium sorption and toxicity in autotrophic biofilms

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Abstract: Autotrophic biofilms (periphyton) accumulate substantial quantities of metals from contaminated water. In this study, we measured the time course of biofilm cadmium sorption, examined the effects of current, biomass, and light on short-term cadmium sorption by biofilms, and tested the toxicity of cadmium to biofilm photosynthesis. The time course of cadmium sorption appeared to be a linear function of time over the 48-h measurement period. Biofilms in current ≥2 $cm \cdot s^{-1}$ sorbed three to five times more cadmium than biofilms in still water. Cadmium sorbed after 4 h was 75% greater in high-biomass biofilm (2.5 mg dry mass·cm^{-2}) than in low-biomass biofilm (0.5 mg dry mass·cm^{-2}), but only in moving water. Light enhanced the sorption of cadmium 40% in one biofilm type. Cadmium toxicity to photosynthesis was evident after 24 h in thin biofilms exposed to initial cadmium concentrations ≥10 $\mu g \cdot L^{-1}$; photosynthesis by thicker biofilms was not significantly impaired even at the highest concentration (100 $\mu g \cdot L^{-1}$). Variations in current, biofilm biomass, and light are likely to influence the movement of metals in flowing systems.

Résumé : Les films biologiques autotrophes (périphyton) accumulent des quantités importantes de métaux dans les eaux contaminées. Dans cette étude, nous avons mesuré la sorption du cadmium par des films biologiques en fonction du temps, examiné les effets du courant, de la biomasse et de la lumière sur la sorption du cadmium par ces films biologiques à court terme, et les effets toxiques du cadmium sur la photosynthèse dans ces films biologiques. Dans notre période de mesure de 48 h, la sorption du cadmium augmentait de façon linéaire en fonction du temps. Les films biologiques exposés à un courant de 2 $cm \cdot s^{-1}$ ou plus sorbaient trois à cinq fois plus de cadmium que les films qui se trouvaient en eau calme. Les films biologiques à forte biomasse (2,5 mg poids sec·cm^{-2}) ont sorbé après 4 h 75% de plus de cadmium que les films à faible biomasse (0,5 mg poids sec·cm^{-2}), mais seulement en eau vive. La lumière a accru la sorption du cadmium de 40% dans un type de film biologique. On a observé que le cadmium était toxique à l'égard de la photosynthèse après 24 h dans les films biologiques minces exposés à des concentrations initiales de cadmium ≥10 $\mu g \cdot L^{-1}$; la photosynthèse dans les films plus épais n'a pas été significativement affectée, même à la plus forte concentration (100 $\mu g \cdot L^{-1}$). Les variations du courant, de la biomasse des films biologiques et de la lumière peuvent probablement influer sur la circulation des métaux dans les systèmes fluviaux.

[Traduit par la Rédaction]

Introduction

Microalgae possess a tremendous capacity for accumulating dissolved metals. Phytoplankton and biofilms (periphyton) bioconcentrate cadmium and mercury more than four orders of magnitude above concentrations in the surrounding water (Hart and Scaife 1977; Hill et al. 1996), and microalgae in the laboratory can accumulate 1–10% of their dry mass as aluminum, tin, zinc, and manganese (Wong et al. 1984; Bender et al. 1994). The propensity of microalgae to bioconcentrate is important because many environmental toxicants that biomagnify to high concentrations in upper trophic levels first enter the food web at the autotroph level. Microalgae may also play an important role in the transport of metals through lotic ecosystems. Autotrophic biofilms cover virtually every exposed substrate in streams, presenting a potentially large sink for metals introduced into streams. The potential to remove dissolved metals from the water column makes autotrophic biofilms attractive for the development of biological systems to treat industrial and domestic waste water (e.g., Wilde and Benemann 1993).

Factors influencing the sorption of dissolved metals by autotrophic biofilms are not well researched. Effects of various water chemistry parameters (e.g., pH, phosphate) on metal sorption have been studied (Genter 1996), and efforts to describe the sorption dynamics of metals onto living and dead algal biomass have been made (Crist et al. 1988; Sloof et al. 1995). However, the effects of basic environmental parameters such as biofilm thickness, water flow, and light have received little research effort. These factors shape the architecture and ecophysiology of biofilms and are likely to influence biofilm metal sorption. Biofilm thickness affects the microenvironment and metabolic activity of cells composing the biofilm (Kuhl et al. 1996). It also affects the ratio of cells on the surface of the biofilm to cells in underlying layers. Rose and Cushing (1970) reported that zinc sorbed only to surface cells and suggested that metal sorption is a

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simple function of total surface area, unrelated to biofilm mass. Flowing water reduces the thickness of the diffusive boundary layer overlying a biofilm, allowing faster replenishment of ions taken up from the boundary layer (Riber and Wetzel 1987). Flowing water may also cause advective currents to penetrate the boundary layer (Gunderson and Jorgensen 1990), increasing delivery rates of metals to biofilms. Light should stimulate metal sorption by biofilms through its effect on photosynthesis. Photosynthesis by autotrophic biofilms provides energy for active transport of metal ions into cells (Pawlik and Skowronski 1994), and it increases pH both in the diffusive boundary layer and within the biofilm matrix itself (Jorgensen et al. 1983; Kuenen et al. 1986; Hartley et al. 1996). Because sorption of metals is strongly influenced by pH (Genter 1996), photosynthetically induced changes in pH within or near the biofilm could have a signficant impact on metal sorption. Cadmium sorption by free-floating microalgae has been reported to be greater in the light (Ting et al. 1989; Pawlik and Skowronski 1994; Subramanian et al. 1994).

Biofilm thickness, water flow, and light vary substantially within and between streams. Understanding the potential influence of these factors on metal sorption is crucial to predicting the movement of metals through flowing-water ecosystems (Stewart et al. 1993). In this study, we examined the effects of biofilm thickness, water flow, and light on cadmium sorption by autotrophic biofilms in the laboratory. We first characterized the time course of cadmium sorption by biofilms and then performed two manipulative experiments that tested the effects of biofilm thickness, current, and light. Potential toxicity of cadmium was examined by measuring biofilm photosynthesis over a logarithmic gradient of cadmium concentrations.

Materials and methods

Sorption dynamics

The time course of cadmium sorption was measured in a flow-through laboratory stream. The stream (21 m long and 0.3 m wide) was supplied with water from an unpolluted section of upper First Creek on the Oak Ridge Reservation. Illumination was provided by metal halide lamps overhanging the stream; irradiances were 50–100 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The experimental stream was kept dry until the day before the sorption measurements began to keep biofilm development minimal. Discharge during the measurements was 0.25 $L \cdot s^{-1}$, resulting in an average velocity of approximately 6 $cm \cdot s^{-1}$ and a stream depth of approximately 1.5 cm. Temperature in the stream at the start of the experiment was 19°C, pH was 8.0, and alkalinity was 139 mg $CaCO_3 \cdot L^{-1}$.

Ceramic tiles (2.4 × 2.4 cm) with attached biofilm were transferred into the stream 2 h before the experiment began. The biofilm was grown on the tiles in an adjacent laboratory stream (supplied by the same water as the experimental stream) and was composed of diatoms (*Achnanthes*, *Synedra*, *Navicula*, and *Cymbella*) and a filamentous cyanophyte (*Lyngbya*). Standing dry mass of the biofilm was 4.85 ± 0.15 $mg \cdot cm^{-2}$ (mean ± SE). Cadmium chloride from a concentrated stock solution was added to the stream with a peristaltic pump positioned 3 m upstream of the biofilm-covered tiles. An array of small ceramic cylinders located between the pump and the tiles created a turbulent zone that mixed the stock solution with the stream water. The effectiveness of mixing was checked with dye before the experiment. Stock solution was added to the stream at a rate that resulted in a cadmium concentration of 1 $\mu g \cdot L^{-1}$ after dilution with stream water. The solution was spiked with ^{109}Cd as a radiotracer for cadmium; ^{109}Cd activity after dilution with stream water was 71 $Bq \cdot L^{-1}$. Stream water ^{109}Cd activity varied little during the experiment: the coefficient of variation of ^{109}Cd activity was 7% in water samples taken at 1, 2, 4, 8, 24, 32, and 48 h after the peristaltic pump was turned on.

Three sets of biofilm-covered tiles (each set consisted of four connected tiles) were removed 10 and 30 min and 1, 2, 4, 8, 24, 32, and 48 h after the peristaltic pump was turned on. The biofilm from each set of harvested tiles was dislodged with a stencil brush, rinsed with distilled water into an aluminum weighing pan, dried at 60°C, and weighed. The amount of ^{109}Cd in the dried biofilm in the pan was determined by gamma spectroscopy using a coaxial, high-purity, intrinsic germanium detector coupled to a Nuclear Data® microprocessor programmed to acquire data in 4096 channels. Full-width half-maximum was 2 keV for the 1332-keV ^{60}Co photon energy. Counting efficiency (8.3%) was determined by adding a known amount of ^{109}Cd to wet biofilm in an aluminum weighing pan, drying the biofilm, and counting the sample.

Biomass and flow effects

Cadmium sorption by different biomasses of autotrophic biofilm was measured in this experiment to determine if metal sorption is a simple function of surface area or a more complex function involving biofilm thickness. The experiment concurrently examined the effect of water movement on metal sorption. Three different biomass levels (0.5 ± 0.1 (mean ± SE), 1.1 ± 0.1, and 2.5 ± 0.3 mg dry $mass \cdot cm^{-2}$) of autotrophic biofilms were obtained by allowing pure cultures of *Stigeoclonium tenue* to grow for different periods of time on the ceramic tiles. This filamentous alga was obtained from the Culture Collection of Algae at the University of Texas at Austin and was grown in soil water amended with 1.8 mM concentrations of $NaHCO_3$, K_2HPO_4, $NaNO_3$, and Tris buffer (pH = 7.2). The three biomass levels corresponded to growing periods of 8, 10, and 12 weeks.

One tile of each biomass level (three tiles in total) were placed in each of six glass crystalizing dishes (12.5 cm in diameter, 6.5 cm high) that contained 250 mL of water from the laboratory stream (pH = 7.8, alkalinity = 96 mg $CaCO_3 \cdot L^{-1}$). Three of the dishes were stirred with a magnetic stir bar to create a current of approximately 6 $cm \cdot s^{-1}$ at the outer edge of the dish where the tiles were placed. The other three dishes were not stirred. The dishes were located in a large environmental chamber (80 cm wide, 120 cm high, 185 cm long) and were illuminated with a combination of incandescent and cool-white fluorescent bulbs at an irradiance of 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Air temperature in the chamber was maintained at 21°C.

Cadmium chloride from a stock solution and ^{109}Cd were added to each dish in quantities to achieve an initial cadmium concentration of 10 $\mu g \cdot L^{-1}$ and an initial radioactivity of 40.4 $kBq \cdot L^{-1}$. Irradiance and temperature during the incubations were 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and 21°C. After 4 h, the tiles with attached biofilms were removed from each dish, immersed for a few seconds in stream water to remove unsorbed ^{109}Cd, and dried at room temperature for 72 h. Gamma spectroscopy quantified the ^{109}Cd sorbed by the biofilm-covered tiles. Counting efficiency (11%) was determined placing a known amount of ^{109}Cd on tiles covered with biofilm, drying the biofilm, and counting the samples. Water samples (1 mL) were also taken from the dishes at the end of the experiment and analyzed with gamma spectroscopy to determine the concentration of cadmium remaining in the dishes. Counting efficiency (10%) for water samples was determined by adding a known amount of ^{109}Cd to a sample vial.

Current gradient and light

Six current velocities (0, 2, 4, 6, 10, and 18 $cm \cdot s^{-1}$) were estab-

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lished in this experiment that simultaneously examined the effect of light on cadmium sorption. The six current velocities were created in 12 crystalizing dishes by varying the speed at which stir bars revolved in the dishes. Each current velocity was replicated in two dishes. Current velocity in each chamber was set by precalibrating the speed knob of the stirrer beneath each dish against current velocity that was quantified by timing the speed at which introduced particles moved around the outer edge of the dish (where tiles were placed). One dish of each current velocity was completely wrapped in aluminum foil to eliminate light.

Biofilms from two different sources were used in this experiment. Both biofilms were grown on ceramic tiles in streams on the Oak Ridge Reservation; biofilm on one set of tiles was grown in Walker Branch (for a description of this stream, see Mulholland et al. 1991), and the other biofilm was grown on tiles in East Fork Poplar Creek (EFPC) (for a description of this stream, see Hill et al. 1996). The biofilm from Walker Branch was dominated by *Stigeoclonium* basal cells and averaged 0.76 ± 0.08 mg dry mass·cm^{-2} (mean ± SE). The biofilm from EFPC was a mixed community of *Oscillatoria, Stigeoclonium*, and small diatoms (e.g., *Nitzschia, Surirella, Navicula, Gomphonema*); it averaged 4.24 ± 0.35 mg dry mass·cm^{-2}. One tile of each biofilm type was added to each dish, which contained 250 mL of laboratory stream water (pH = 8.2, alkalinity = 112 mg $CaCO_3$·L^{-1}). Cadmium chloride and ^{109}Cd were added in quantities to achieve an initial cadmium concentration of 10 μg·L^{-1} and an initial radioactivity of 99.2 kBq·L^{-1}. The biofilm-covered tiles were removed after 4 h, and ^{109}Cd sorbed by the tiles was measured as before. Water samples (1 mL) were taken from the dishes at the end of the experiment to determine the final concentration of cadmium in the water. The experiment was performed twice, on consecutive days.

Toxicity test

Potential toxic effects of cadmium on autotrophic biofilms were investigated in two experiments. Five cadmium concentrations were used in each of these experiments: 0, 0.1, 1, 10, and 100 μg·L^{-1}. These concentrations were chosen to include concentrations expected in both uncontaminated (<1 μg·L^{-1}) and contaminated (≥1 μg·L^{-1}) habitats. Each concentration was replicated three times in separate crystallizing dishes containing 250 mL of stream water (pH = 8.2, alkalinity = 137 mg $CaCO_3$·L^{-1}). The first experiment exposed biofilms to cadmium for 4 h; the second experiment exposed biofilms for 26 h. Three different biomasses of biofilms were used in both experiments: low-biomass biofilms grown in the laboratory streams, medium-biomass biofilms grown in EFPC, and high-biomass biofilms grown in EFPC. Differences between medium- and high-biomass biofilms were due to different development times in EFPC. The low-biomass biofilm was composed of *Audouinella, Stigeoclonium*, and diatoms (*Cocconeis, Fragilaria*). The medium- and high-biomass biofilms from EFPC were composed of *Stigeoclonium, Phormidium*, and diatoms (*Nitzschia, Achnanthidium, Fragilaria, Gomphonema, Navicula*). The dry masses of the low-, medium-, and high-biomass biofilms were 0.89 ± 0.05 (mean ± SE), 2.24 ± 0.11, and 2.98 ± 0.17 mg·cm^{-2}, respectively. One tile of each biofilm type was added to each crystalizing dish.

Photosynthesis was the response variable in both experiments. It was measured by ^{14}C uptake. In the 4-h experiment, ^{14}C was added at the beginning of the experiment. In the 26-h experiment, ^{14}C was added 24 h after cadmium had been added. Each crytallizing dish received 148 kBq of $NaH^{14}CO_3$. At the end of each experiment, tiles with attached biofilms were removed from the crystallizing dishes, rinsed briefly in stream water to remove unincorporated ^{14}C, and placed in extraction jars containing dimethyl sulfoxide for the extraction of chlorophyll *a* and ^{14}C-labeled photosynthate (Palumbo et al. 1987). Extracted chlorophyll *a* was measured spectrophotometrically, and the ^{14}C in the labeled photosynthate was assayed via liquid scintillation. A more detailed description of the ^{14}C methodology is given in Hill and Boston (1991).

Results

Cadmium sorption dynamics

Cadmium sorbed by biofilm increased steadily over the 48-h period of sampling (Fig. 1). Linear regression fit the data well (r^2 = 0.99), even at early sampling times. Sorption may have been nonlinear before 10 min, but the amount of cadmium sorbed in this period was very small (<10% of the amount sorbed over 2 h, <1% of the amount sorbed over 48 h).

Biomass and flow effects

Cadmium sorption increased with both biofilm biomass and water movement (Fig. 2). The effect of biomass appeared to depend on flow: cadmium sorption in moving water increased approximately 75% as biofilm biomass increased from 0.5 and 2.5 mg dry mass·cm^{-2}, whereas the sorption increase with biomass was quite small in still water. Regression through the sorption data from the containers with moving water resulted in a slope significantly greater than zero (P = 0.002), whereas regression through the data from the containers with still water resulted in a slope estimate that did not differ significantly from zero (P = 0.30). The effects of current were strong for all biomasses: cadmium sorption was three to five times greater in moving water than it was in still water. Cadmium concentrations at the end of the experiment were 5.0 ± 0.1 μg·L^{-1} (mean ± SE) in the dishes with still water and 3.1 ± 0.1 μg·L^{-1} in the dishes with moving water (initial cadmium = 10 μg·L^{-1}). Although depletion of cadmium to 3–5 μg·L^{-1} may have constrained uptake to some extent by the end of the experiment, it would have constrained all biomass treatments equally because they were all represented in each container.

Current gradient and light

Cadmium sorption was again strongly influenced by current. Sorption in both dark and illuminated chambers increased dramatically as current velocity increased from 0 to 2 cm·s^{-1} (Fig. 3). Velocities >2 cm·s^{-1} did not appear to further increase cadmium sorption by Walker Branch biofilm. Mean cadmium sorbed after 4 h by Walker Branch biofilm was 14.1 ± 2.9 ng·cm^{-2} (mean ± SE) at zero current and 59.0 ± 6.0 ng·cm^{-2} at current ≥2 cm·s^{-1}. Sorption by EFPC biofilm increased slightly as velocity increased above 2 cm·s^{-1}. Mean cadmium sorbed by EFPC biofilm after 4 h was 13.2 ± 2.2 ng·cm^{-2} (mean ± SE) at zero current and 67.5 ± 6.5 ng·cm^{-2} at current ≥2 cm·s^{-1}. Cadmium concentrations at the end of the experiment were 7.7 ± 0.2 μg·L^{-1} (mean ± SE) in dishes with still water and 3.3 ± 0.3 μg·L^{-1} in dishes with moving water (current velocities ≥2 cm·s^{-1}).

Light effects depended on biofilm type. Cadmium sorption by Walker Branch biofilm was consistently higher in illuminated chambers than in darkened chambers at all current velocities. The average increase in sorption caused by light in this biofilm was approximately 40%. Differences between light and dark treatments were generally smaller and less consistent for EFPC biofilm.

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Fig. 1. Time course of cadmium sorption by autotrophic biofilms. Sorption data are expressed as the quantity of cadmium absorbed per unit of substrate area. Each point is the mean concentration of cadmium sorbed by three replicated sets of tiles. Inset illustrates sorption over the first 2 h. Error bars represent ±1 SE; some error bars are covered by the mean points. The equation for the regression line is $y = 0.99x + 0.24$ ($r^2 = 0.99$, $P < 0.001$).

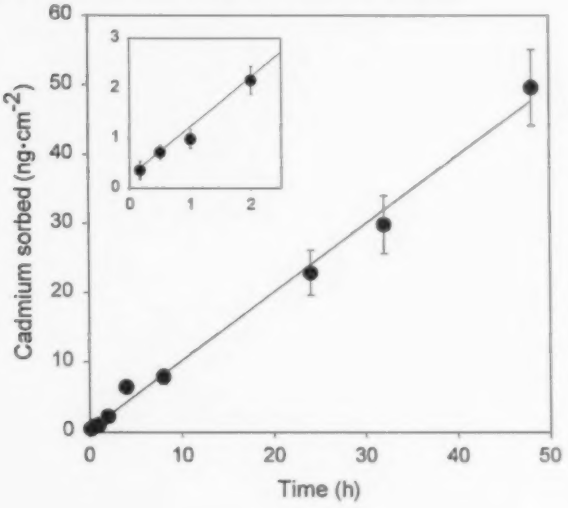
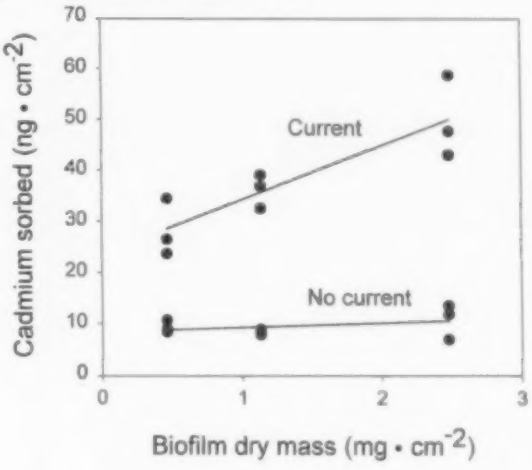


Fig. 2. Biomass and flow effects on the sorption of cadmium by autotrophic biofilms. Data represent the mass of cadmium accumulated per unit of substrate area after 4 h. Current = 6 cm·s^{-1}. The regression line through current data is $y = 11x + 24$ ($r^2 = 0.74$, $P = 0.002$); the regression line through the no current data is $y = 0.93x + 8.3$ ($r^2 = 0.15$, $P = 0.3$).



Cadmium toxicity

The autotrophic biofilms were relatively resistant to cadmium toxicity. After 4 h of exposure, photosynthesis was not significantly impaired at any cadmium concentration, although photosynthesis by the low-biomass biofilm may have been verging on impairment at 100 μg·L^{-1} (Fig. 4). After 26 h, photosynthesis by the high-biomass biofilm remained unaffected by cadmium, photosynthesis by the medium-biomass biofilm was slightly (but not significantly) diminished by increasing cadmium concentration, and photosynthesis by the low-biomass biofilm declined steadily at concentrations ≥1 μg·L^{-1} (Fig. 4).

Discussion

Sorption dynamics

Metal sorption by microalgae is often characterized by an initial phase of rapid sorption followed by a second phase of slower uptake that gradually approaches an asymptote after a few to many hours (e.g., Genter et al. 1988; Reinfelder and Chang 1999). In the biphasic model of metal uptake that describes these dynamics, metal ions bind to and saturate negatively charged sites (e.g., carboxylic groups) on the exterior of cells in the first phase and are then transported to the interior of the cell via energy-dependent processes in the second phase (Xue et al. 1988; Ting et al. 1989). The time course of cadmium sorption by our biofilm exhibited little evidence of a significant first phase, suggesting that internal uptake was the most important component of cadmium accumulation. The linear sorption dynamics that we observed may be due to a comparatively low, but environmentally realistic, cadmium concentration that allowed active transport to keep pace with adsorption. Biphasic sorption of cadmium by phytoplanktonic diatoms became more linear as cadmium concentration decreased (Conway and Williams 1979), and accumulation of cadmium at <0.1 μg·L^{-1} by lake periphyton did not exhibit a significant rapid uptake phase (Stephenson and Turner 1993).

Current

Current had a large stimulatory effect on cadmium sorption by biofilms in our study, increasing sorption three to five times over that in still water. Several processes may account for the effect of current. First, current decreases the thickness of the diffusive boundary layer above biofilms. Metal concentrations in the diffusive boundary layer should be lower than those in the overlying bulk water because of metal uptake by the biofilm. Uptake should create a vertical zone of ion depletion through which new ions must diffuse from the bulk water to reach the biofilm. Reduction in the thickness of this layer could directly increase the rate at which cadmium ions move to the biofilm. Second, current may increase ion delivery to biofilms by increasing the frequency of advective currents that penetrate the boundary layer and biofilm (Gunderson and Jorgensen 1990). Third, heterogeneities in biofilm architecture may allow current to penetrate into the biofilm matrix (deBeer et al. 1994), allowing delivery of metal ions to subsurface cells. Fourth, current may increase cadmium sorption indirectly by stimulating biofilm metabolism. Metabolic processes such as photosynthesis, respiration, and nutrient uptake all occur at higher rates in moving water because metabolic substrates move to the biofilm faster and metabolic byproducts are moved away faster (Riber and Wetzel 1987). However, increases in biofilm photosynthesis cannot explain the stimulatory effect of water flow on cadmium sorption in our experiments because

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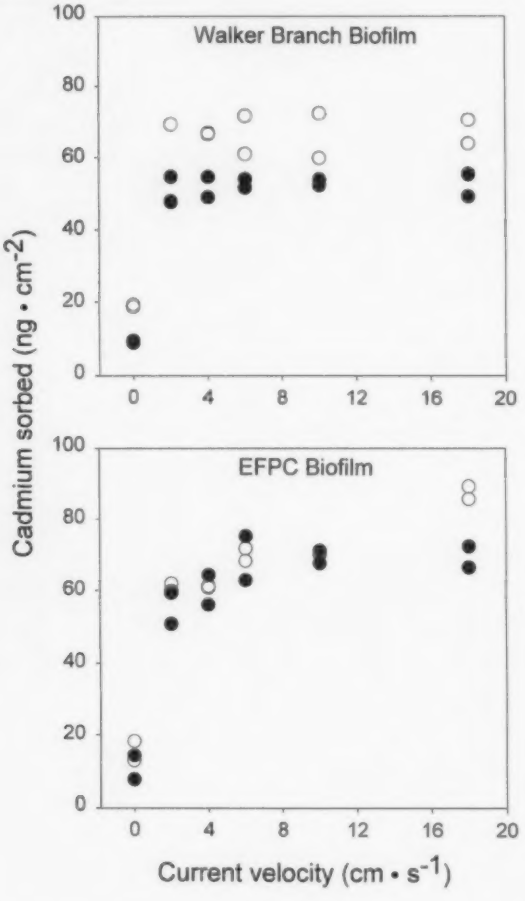
Fig. 3. Flow and light effects on the sorption of cadmium by autotrophic biofilms. Data represent the mass of cadmium accumulated per unit of substrate area after 4 h. Data points are from individual sorption chambers. Solid circles, dark chambers; open circles, light chambers.



sorption by biofilms in the dark also increased with water flow.

Increases in current beyond 2 $cm \cdot s^{-1}$ had little additional effect on cadmium sorption. This apparent saturation at low current velocity may have been in part due to depletion of dissolved cadmium in the stirred containers. Cadmium concentrations in dishes with moving water were reduced from 10 to <4 $\mu g \cdot L^{-1}$ by the end of the experiment. These lower concentrations may have slowed sorption rates in these containers and caused a flattening in our sorption versus current response. No quantitative analysis of the effect of current on metal sorption has been published in the literature, but the effect of current on biofilm metabolism plateaus at velocities ranging from 5.4 to 50 $cm \cdot s^{-1}$ (Whitford 1956; Lock and John 1979; Horner et al. 1990). The specific relationship between sorption and current velocity may depend on the total mass or density of biofilms, particularly if horizontal flow through the biofilm is an important vector of ions to interior sorption sites. Regardless of the precise shape of the sorption versus current curve, small current velocities clearly had a large influence on cadmium uptake by our biofilms.

Light

The effect of light on cadmium sorption varied between biofilm types. Light increased sorption 40% by Walker Branch biofilm, but its effect on EFPC biofilm was inconsistent. Other studies report strong and positive effects of light on the sorption of metals by phytoplankton (Ting et al. 1989; Pawlik and Skowronski 1994; Skowronski et al. 1998) and autotrophic biofilms (Gray and Hill 1995), so the inconsistent response to light by EFPC biofilm was somewhat surprising. It is unclear if this response is an anomaly unique to the specific EFPC biofilms used in this study or if these biofilms represent a more general subset of biofilms that do not respond to light.

When light does increase metal uptake by microalgae, its effect is presumed to be mediated through photosynthesis. Darkness and photosynthetic inhibitors such as 3′-(3,4-dichlorophenyl)-1′,1′-dimethyl urea similarly reduce cadmium sorption by the cyanobacteriium *Synechocystis aquatilis* (Pawlik and Skowronski 1994). Biofilm sorption of nickel over a gradient of light intensities closely matches carbon uptake rates over the same gradient, suggesting a quantitative link to photosynthesis (Gray and Hill 1995). Pawlik and Skowronski (1994) and Skowronski et al. (1998) argued that photosynthesis increases cadmium sorption because it provides the metabolic energy required for the metal's active transport into algal cells. Light/photosynthetic effects may also operate external to algal cells in biofilms. Photosynthesis can raise pH several units in the water immediately surrounding microautotrophs, making protons relatively scarce in the diffusive boundary layer and within the biofilm itself. Positively charged metals such as cadmium compete with protons for negatively charged binding sites (Crist et al. 1988), so adsorption of cadmium at these sites increases with increasing pH (e.g., Skowronski 1986; Sampedro et al. 1995). Increases in pH due to photosynthesis can also cause the precipitation of calcite ($CaCO_3$) at the biofilm surface or within the biofilm (Hartley et al. 1996), where pH can exceed 9 (Jorgensen et al. 1983; Kuenen et al. 1986). Cadmium binds strongly to precipitating calcite particles (Cicerone et al. 1999).

Biomass

Increasing cadmium sorption with increasing biofilm biomass implies a significant contribution of subsurface cells to metal sorption. These cells may contribute directly to sorption by providing additional sorption sites, or they may contribute indirectly by providing additional photosynthetic units that raise pH. The implication of a three-dimensional sorption process is that streams or water treatment channels containing thick biofilms will be more effective at removing metals than streams or channels with thin biofilms. However, the effectiveness of additional biofilm biomass in cadmium sorption may be limited to flowing water, since the effect was evident only in chambers that were stirred in our experiment. In the absence of current, diffusion of cadmium into cells deep in the biofilm matrix may be so slow that effects of biomass can only be detected over periods longer than 4 h.

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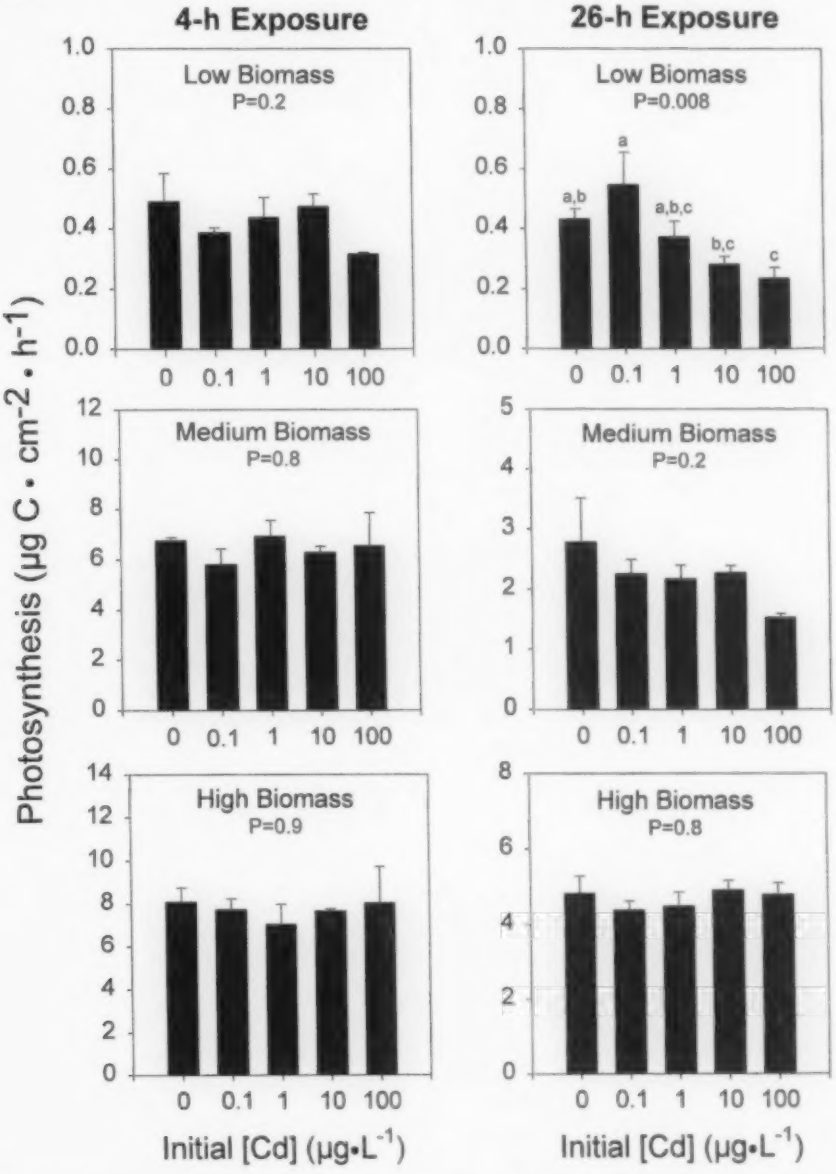
Fig. 4. Cadmium effects on photosynthesis by low-, medium-, and high-biomass biofilms. Letters above bars in the top right graph indicate results of Fisher's protected least significant difference multiple comparison procedure; bars with the same letter are not significantly different ($P > 0.05$).



Toxicity of cadmium to biofilms

The biofilms used in our experiments appeared to be relatively resistant to the toxicity of cadmium. Photosynthetic impairment was detected only in the 26-h exposure, and only in the low-biomass biofilm. Toxicity has been observed at cadmium concentrations <100 $\mu g \cdot L^{-1}$ in a variety of microalgae (Peterson et al. 1984; Lawrence et al. 1989; Leborans and Novillo 1996) and at concentrations as low as 2 $\mu g \cdot L^{-1}$ in natural phytoplankton (Conway 1978; Nalewajko 1995). These effects were generally observed after exposures lasting for several days. In our study, photosynthetic impairment was statistically significant only in the longer of the two toxicity experiments, suggesting a significant lag between sorption and toxicity. This lag may result from the time required for cadmium ions to accumulate to toxic levels inside the cell, where metabolic disruption occurs. Some of the lag may also be due to slow diffusion of cadmium ions through the entire three-dimensional biofilm matrix.

The greater sensitivity of the thin biofilms to cadmium suggests that additional biomass in thicker biofilms mitigates the toxicity of cadmium in some way. Additional biomass sorbs more cadmium, effectively reducing the concentration

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of cadmium in the water and reducing cadmium available per cell in the biofilm. The diluting effect of biomass should be effective in reducing toxicity as long as the sorption capacity of the biofilm is not reached. Biomass may not mitigate toxicity in contaminated streams where a steady supply of new cadmium ions is constantly transported to attached biofilms.

Implications

Our experiments have important implications for the transport and fate of metals in flowing-water systems. Extrapolation of our results to natural streams suggests that natural variability in biofilm biomass, light, and current will affect in the movement of metals through these ecosystems. Streams or stream sections with higher standing crops of biofilms should retain a higher proportion of dissolved metals near the metals' point of entry. In terms of solute spiralling, this means that the average "uptake distance" for metals will be reduced in streams where biofilm thickness is greater due to higher biofilm productivity or lower losses from grazing or scour (e.g., Mulholland et al. 1983). Flowing water obviously transports metals downstream, but this downstream movement should be offset at least partially by the very large increase in sorption rate stimulated by current. Metal sorption by biofilms may be significantly slower in pools where current velocities are nearly zero. It should also be slower in biofilms growing in the shade of terrestrial vegetation and at depths where light has been attenuated by the overlying water column.

Our results can also be applied to the design of biofilm-based water treatment systems. Artificial stream channels constructed to treat industrial or domestic waste water contaminated with metals should allow the development of at least moderate thicknesses of autotrophic biofilm. The time course of cadmium sorption indicates that moderately thick biofilms can be used to remove dilute concentrations of contaminants for at least 48 h without reaching saturation. This biofilm should be illuminated during waste water treatment to encourage photosynthetically related sorption, and current should be provided to increase the efficiency of metal sorption. Biofilms in engineered systems should be relatively tolerant of cadmium concentrations up to 100 $\mu g \cdot L^{-1}$ for at least 4 h, unless cadmium-sensitive microalgal species dominate the biofilm.

Autotrophic biofilms are expected to be important reservoirs for contaminants because they cover virtually every exposed surface in aquatic ecosystems. Biofilm sorption processes should be especially important in streams where the ratio of streambed area to water volume is relatively large. Because of the ubiquitous distribution, sorptive nature, and trophic contributions of autotrophic biofilms, variations in the environmental factors that affect the biofilm sorptive properties could significantly impact the movement of contaminants in the water column and through the food web.

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References

Bender, J., Gould, J.P., Vatcharapijarn, Y., Young, J.S., and Phillips, P. 1994. Removal of zinc and manganese from contaminated water with cyanobacteria mats. Water Environ. Res. **66**: 679–683.

Cicerone, D.S., Stewart, A.J., and Roh, Y. 1999. Diel cycles in calcite production and dissolution in an eutrophic basin. Environ. Toxicol. Chem. **18**: 2169–2177.

Conway, H.L. 1978. Sorption of arsenic and cadmium and their effects on growth, micronutrient utilization, and photosynthetic pigment composition of *Asterionella formosa*. J. Fish. Res. Board Can. **35**: 286–294.

Conway, H.L., and Williams, S.C. 1979. Sorption of cadmium and its effect on growth and the utilization of inorganic carbon and phosphorus of 2 freswater diatoms. J. Fish. Res. Board Can. **36**: 579–586.

Crist, R.H., Oberhoiser, K., Schwartz, D., Marzoff, J., and Ryder, D. 1988. Interactions of metals and protons with algae. Environ. Sci. Technol. **22**: 755–760.

deBeer, D., Stoodley, P., and Lewandowski, Z. 1994. Liquid flow in heterogeneous biofilms. Biotechnol. Bioeng. **44**: 636–641.

Genter, R.B. 1996. Ecotoxicology of inorganic chemical stress to algae. *In* Algal ecology, freshwater benthic ecosystems. *Edited by* R.J. Stevenson, M.L. Bothwell, and R.L. Lowe. Academic Press, San Diego, Calif. pp. 403–468.

Genter, R.B., Colwell, F.S., Pratt, J.R., Cherry, D.S., and Cairns, J., Jr. 1988. Changes in epilithic communities due to individual and combined treatments of zinc and snail grazing in stream mesocosms. Toxicol. Ind. Health, **4**: 185–201.

Gray, B.R., and Hill, W.R. 1995. Nickel sorption and photosynthetic activity of periphyton. J. North Am. Benthol. Soc. **14**: 299–305.

Gunderson, J.K., and Jorgensen, B.B. 1990. Microstructure of diffusive boundary layers and the oxygen uptake of the sea floor. Nature (Lond.), **345**: 604–607.

Hart, B.A., and Scaife, B.D. 1977. Toxicity and bioaccumulation of cadmium in *Chlorella pyrenoidosa*. Environ. Res. **14**: 401–413.

Hartley, A.M., House, W.A., Leadbeater, B.X.C., and Callow, M.E. 1996. The use of microelectrodes to study the precipitation of calcite upon algal biofilms. J. Colloid Interface Sci. **183**: 498–505.

Hill, W.R., and Boston, H.L. 1991. Community development alters photosynthesis–light relations in stream periphyton. Limnol. Oceanogr. **36**: 1375–1389.

Hill, W.R., Stewart, A.J., and Napolitano, G.E. 1996. Mercury speciation and bioaccumulation in lotic primary producers and primary consumers. Can. J. Fish. Aquat. Sci. **53**: 812–819.

Horner, R.R., Welch, E.B., Seeley, M.R., and Jacoby, J.M. 1990. Response of periphyton to changes in current velocity, suspended sediment, and phosphorus concentration. Freshwater Biol. **24**: 215–232.

Jorgensen, B.B., Revsbech, N.P., and Cohen, Y. 1983. Photosynthesis and structure of benthic microbial mats: microelectrode and SEM studies of four cyanobacterial communities. Limnol. Oceanogr. **28**: 1075–1093.

Kuenen, J.G., Jorgensen, B.B., and Revsbech, N.P. 1986. Oxygen microprofiles of trickling filter biofilms. Water Res. **20**: 1589–1598.

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Kuhl, M., Glud, R.N., Ploug, H., and Ramsing, N.B. 1996. Microenvironmental control of photosynthesis and photosynthesis-coupled respiration in an epilithic cyanobacterial biofilm. J. Phycol. **32**: 799–812.

Lawrence, S.G., Holoka, M.H., and Hamilton, R.D. 1989. Effects of cadmium on a microbial food chain, *Chlamydomonas reinhardii* and *Tetrahymena vorax*. Sci. Total Environ. **87/88**: 381–395.

Leborans, G.F., and Novillo, A. 1996. Toxicity and bioaccumulation of cadmium in *Olisthodiscus luteus* (Raphidophyceae). Water Res. **30**: 57–62.

Lock, M.A., and John, P.H. 1979. The effect of flow patterns on uptake of phosphorus by river periphyton. Limnol. Oceanogr. **24**: 376–383.

Mulholland, P.J., Newbold, J.D., Elwood, J.W., and Hom, C.L. 1983. The effect of grazing intensity on phosphorus spiralling in autotrophic streams. Oecologia, **53**: 358–366.

Mulholland, P.J., Steinman, A.D., Palumbo, A.V., Elwood, J.E., and Kirschtel, D.B. 1991. Role of nutrient spiralling and herbivory in regulating periphyton communities in laboratory streams. Ecology, **72**: 966–982.

Nalewajko, C. 1995. Effects of cadmium and metal-contaminated sediments on photosynthesis, heterotrophy, and phosphate uptake in Mackenzie River Delta phytoplankton. Chemosphere, **30**: 1401–1414.

Palumbo, A.V., Mulholland, P.J., and Elwood, J.W. 1987. Simultaneous measurements of periphyton production, chlorophyll, and ATP using DMSO extraction. Limnol. Oceanogr. **32**: 464–471.

Pawlik, B., and Skowronski, T. 1994. Transport and toxicity of cadmium: its regulation in the cyanobacterium *Synechocystis aquatilis*. Environ. Exp. Bot. **34**: 225–233.

Peterson, H.G., Healey, F.P., and Wagemann, R. 1984. Metal toxicity to algae: a highly pH dependent phenomenon. Can. J. Fish. Aquat. Sci. **41**: 974–979.

Reinfelder, J.R., and Chang, S.I. 1999. Speciation and microalgal bioavailability of inorganic silver. Environ. Sci. Technol. **33**: 1860–1863.

Riber, H.H., and Wetzel, R.G. 1987. Boundary layer and internal-diffusion effects on phosphorus fluxes in lake periphyton. Limnol. Oceanogr. **32**: 1181–1194.

Rose, F.L., and Cushing, C.E. 1970. Periphyton: autoradiography of zinc-65 adsorption. Science (Washington, D.C.), **168**: 576–577.

Sampedro, M.A., Blanco, A., Llama, M.J., and Serra, J.L. 1995. Sorption of heavy metals to *Phormidium laminosum* biomass. Biotechnol. Appl. Biochem. **22**: 355–366.

Skowronski, T. 1986. Adsorption of cadmium on green microalga *Stichococcus bacillaris*. Chemosphere, **15**: 77–79.

Skowronski, T., De Knecht, J.A., Simons, J., and Verkleij, J.A.C. 1998. Phytochelatin synthesis in response to cadmium uptake in *Vaucheria* (Xanthophyceae). Eur. J. Phycol. **33**: 87–91.

Sloof, J.E.,Viragh, A., and Der Veer, B.V. 1995. Kinetics of cadmium uptake by green algae. Water Air Soil Pollut. **83**: 105–122.

Stephenson, M., and Turner, M.A. 1993. A field study of cadmium dynamics in periphyton and in *Hyalla azteca* (Crustacea: Amphipoda). Water Air Soil Pollut. **68**: 341–361.

Stewart, A.J., Hill, W.R., and Boston, H.L. 1993. Grazers, periphyton, and toxicant movement in streams. Environ. Toxicol. Chem. **12**: 955–957.

Subramanian, V.V., Sivasubramanian, V., and Gowrinathan, K.P. 1994. Uptake and recovery of heavy metals by immobilized cells of *Aphanocapsa pulchra* (Kutz) Rabenh. J. Environ. Sci. Health Part A. Environ. Sci. Eng. **29**: 1723–1733.

Ting, Y.P., Lawson, F., and Prince, I.G. 1989. Uptake of cadmium and zinc by the alga *Chlorella vulgaris*: Part 1. Individual ions species. Biotechnol. Bioeng. **34**: 990–999.

Whitford, L.A. 1956. The communities of algae in the springs and spring streams of Florida. Ecology, **37**: 433–442.

Wilde, E.W., and Benemann, J.R. 1993. Bioremoval of heavy metals by the use of microalgae. Biotech. Adv. **11**: 781–812.

Wong, P.T.S., Maguire, R.J., Chau, Y.K., and Kramar, O. 1984. Uptake and accumulation of inorganic tin by a freshwater alga, *Ankistrodesmus falcatus*. Can. J. Fish. Aquat. Sci. **41**: 1570–1574.

Xue, H.-B., Stumm, W., and Sigg, L. 1988. The binding of heavy metals to algal surfaces. Water Res. **22**: 917–926.

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Factors influencing the growth of *Mougeotia* in experimentally acidified mesocosms

Jennifer L. Klug and Janet M. Fischer

Abstract: Acidification causes profound changes in species composition in aquatic systems. We conducted mesocosm experiments in three northern Wisconsin lakes (Trout Lake, Little Rock - Reference, Little Rock - Treatment) to test how different phytoplankton communities respond to acidification. Major differences exist among these lakes in water chemistry and phytoplankton community composition. In each lake, three pH treatments (control, press (sustained pH 4.7), and pulse (alternating pH 4.7 and ambient pH)) were maintained for 6 weeks. We observed a striking increase in species in the genus *Mougeotia* in all systems. *Mougeotia* is a filamentous green alga often found in acidified lakes. The magnitude of the *Mougeotia* increase differed among lakes and treatments, and we used an autoregressive model to identify potential factors responsible for these differences. Our results suggest that biotic factors such as competition with other algae played a relatively minor role in regulating *Mougeotia* dynamics. Instead, pH and abiotic factors associated with changes in pH (e.g., dissolved inorganic carbon) were important predictors of *Mougeotia* dynamics.

Résumé : L'acidification entraîne de profonds changements dans la composition par espèces dans les systèmes aquatiques. Nous avons effectué des expériences en mésocosmes dans trois lacs du nord du Wisconsin (lac Trout, Little Rock - référence, Little Rock - traitement) pour établir comment différentes communautés phytoplanctoniques répondent à l'acidification. Il y a entre ces lacs de nombreuses différences aux chapitres de la chimie de l'eau et de la composition de la communauté phytoplanctonique. Dans chaque lac, nous avons maintenu durant six semaines trois régimes de pH : pH contrôle, pH acide de 4,7 soutenu et alternance entre le pH 4,7 et le pH ambiant. Nous avons observé un accroissement très marqué de l'abondance des espèces du genre *Mougeotia* sous tous les traitements. *Mougeotia* est un genre d'algues vertes filamenteuses souvent présentes dans les lacs acidifiés. Le degré d'accroissement de l'abondance des espèces de ce genre variait selon les lacs et les traitements, et nous avons utilisé un modèle autorégressif pour identifier les facteurs qui pouvaient être responsables de ces différences. Nos résultats laissent penser que les facteurs biotiques, comme la compétition avec d'autres algues, jouaient un rôle relativement mineur dans la régulation de la dynamique de *Mougeotia*. On a plutôt observé que le pH et certains facteurs abiotiques associés aux changements dans le pH (p. ex. le carbone organique dissous) influaient de façon importante sur la dynamique de *Mougeotia*.

[Traduit par la Rédaction]

Introduction

Filamentous green algae from the family Zygnemataceae (including *Mougeotia*, *Spirogyra*, and *Zygogonium*) are widely recognized as indicators of acidification (Turner et al. 1987; Jackson et al. 1990; Webster et al. 1992). These algae are primarily benthic but form metaphytic mats (no longer attached to the substrate) at high density (Howell et al. 1990; Turner et al. 1995*a*). Substantial portions of the shoreline in experimentally or culturally acidified lakes (up to 100%) may be covered by filamentous green algae, which may drastically alter littoral zone processes (Jackson et al. 1990; Turner et al. 1995*a*). In addition, blooms of filamentous green algae can reach nuisance levels in some lakes that are naturally moderately acidic (SPR Assoc. Inc. 1986, cited in France and Welbourn 1992). Thus, limnologists and lake managers have an interest in determining the factors that regulate the growth of filamentous green algae.

Several hypotheses have been suggested to explain the abundance of filamentous green algae at low pH. Very few studies have been able to address the direct physiological effect of acidity on *Mougeotia*, partially because changes in pH are accompanied by changes in other potentially important variables (e.g., dissolved inorganic carbon (DIC) and metals). Müller (1980) suggested that the optimal growth of *Mougeotia* may occur at low pH; however, this study did not distinguish between the effects of decreased pH and decreased DIC. DIC concentrations decrease as pH decreases due to loss of bicarbonate (Stumm and Morgan 1996). At low pH, filamentous green algae (including *Mougeotia*) may have a competitive advantage if they are better able to sequester DIC at lower concentrations than other algae (Jackson et al. 1990; Turner et al. 1991; Graham et al. 1996). Other studies have suggested that the increase in filamentous green algae at low pH could be caused by decreases in herbivory (Turner et al. 1987; Fairchild and Sherman 1992;

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Table 1. Basic limnological characteristics of the study sites.

	Area (ha)	Mean depth (m)	1995 summer Secchi depth (m)	1995 summer pH	1995 summer ANC ($\mu equiv \cdot L^{-1}$)	1995 TP ($\mu g \cdot L^{-1}$)	1995 TN ($\mu g \cdot L^{-1}$)
Trout Lake	1608	14.6	4.3	8.1	829	5.8	244.9
Little Rock - Reference	8.1	3.1	4.9	6.1	31	5.3	263.3
Little Rock - Treatment	9.8	3.9	5.1	5.9	14	0.7	145.1

Note: Area, mean depth, and acid-neutralizing capacity (ANC) data were provided by the North Temperate Lakes Long-Term Ecological Research site (http://limnosun.limnology.wisc.edu). All other data were collected as part of this study at epilimnetic lake stations adjacent to our experiments. Secchi depth and pH values are averages compiled from weekly samples collected from 19 June to 31 July 1995. Total phosphorus (TP) and total nitrogen (TN) samples were collected during the week of 19 June 1995.

Graham et al. 1996). Macrograzers such as snails (Okland 1980; Fairchild and Sherman 1992), crayfish (Schindler et al. 1985), and tadpoles (Vinebrooke 1996; Graham and Vinebrooke 1998) can be less abundant at low pH.

Although filamentous green algae, such as *Mougeotia*, have been well studied in acidified systems, researchers still do not understand why they are rarely dominant at high pH. Numerous studies show that *Mougeotia* is typically only common when pH is <6.0 (e.g., France and Welbourn 1992; Fairchild and Sherman 1993). However, not only can *Mougeotia* survive at high pH, a recent laboratory study showed that a *Mougeotia* species isolated from a metaphytic bloom grew best at pH = 8 (Graham et al. 1996). *Mougeotia* may have a preference for dissolved carbon dioxide, which makes up a smaller percentage of total DIC as pH increases (Jackson et al. 1990). Alternatively, *Mougeotia* may be an inferior competitor for bicarbonate (the dominant form of DIC at higher pH) and (or) nitrogen and phosphorus at high pH (Jackson et al. 1990; Turner et al. 1991; Graham et al. 1996).

Our goal for this paper is to elucidate some of the potential mechanisms that may affect the growth rate of *Mougeotia* at low and high pH. We conducted a mesocosm experiment in three lakes. The experiment was originally designed to test whether different phytoplankton communities showed consistent responses to acidification. We chose three lakes that differed in zooplankton (Fischer 1997) and phytoplankton community composition and in other chemical factors (Table 1) that may affect how phytoplankton respond to low pH. The most consistent response was an increase in *Mougeotia* in all three lakes. The variability among lakes allowed us to use a regression model to see whether the observed changes were due to the pH manipulations or due to other factors that may be important in regulating *Mougeotia*. Furthermore, the different acidification treatments allowed us to separate the effects of pH and DIC on *Mougeotia* population growth. We conducted a "press" manipulation in which pH was experimentally reduced and fixed at a low level. In this treatment, pH and DIC were closely correlated due to the loss of bicarbonate at low pH. The other acidified treatment was a "pulse" manipulation in which pH was dropped for short periods and then raised. Because changes in DIC lagged behind the pulsed changes in pH, this treatment decoupled the normally strong correlation between pH and DIC. The decoupling of DIC and pH was a fortuitous result that, combined with the comparison of three treatments in three lakes, helped us to infer which factors played a role in regulating *Mougeotia* population growth rate at different pH.

Methods

Study sites

We performed a mesocosm experiment in three lakes in northern Wisconsin, U.S.A. (basic limnological characteristics are summarized in Table 1). Trout Lake (46°00′N, 89°40′W) is one of seven lakes in the North Temperate Lakes Long-Term Ecological Research site located in Vilas County, Wisconsin. Nearby Little Rock Lake (45°59′N, 89°42′W) was the site of a whole-lake acidification experiment in the 1980s (Brezonik et al. 1993). In 1984, this seepage lake was divided with a vinyl curtain into two basins (hereafter Little Rock - Reference and Little Rock - Treatment). Little Rock - Treatment was experimentally acidified throughout the 1980s and has been recovering since 1990. Little Rock - Reference was unmanipulated. At the time of our experiment, pH in Little Rock - Treatment was similar to that in Little Rock - Reference (Table 1).

Experimental design

The experiment was conducted during 19 June – 31 July 1995. At each site (Trout Lake, Little Rock - Reference, and Little Rock - Treatment), three control mesocosms remained at ambient pH (8.1, 6.1, and 5.9, respectively) (Fig. 1). Two acidified treatments with three replicates each were employed: press and pulse. In the press treatment, we lowered pH to 4.7 ± 0.2 after initial sampling. In the pulse treatment, we lowered pH to 4.7 ± 0.2 for the first week of the experiment. In the second week, we raised pH to ambient ± 0.2 for 2 weeks. We again lowered pH to 4.7 in the fourth week and then raised it to ambient in the fifth week (Fig. 1). We manipulated pH using 2.0 N sulfuric acid and 1.0 N sodium hydroxide. All bags (including controls) were thoroughly mixed after each manipulation, and pH was measured three times a week. The experiment was terminated after 6 weeks. We did not include a lake control because the goal of the experiment was to explore potential mechanisms involved in phytoplankton responses to acidification rather than to predict whole-lake phenomena.

Mesocosm design

We used nine new polyethylene bags, 0.8 m in diameter and 4.0 m deep (~2000 L), as mesocosms. The bags were open at the top to allow exchange with the atmosphere and closed at the bottom to prevent mixing with lake water. We attached the bags to a floating wooden frame that was oriented in a north–south direction. We stocked phytoplankton, protozoans, and bacteria into each bag by pumping water from 2 m depth through an 80-μm-mesh screen to exclude most crustacean zooplankton. We then restocked crustacean zooplankton at ambient lake density by taking net tows with an 80-μm net. Tows were taken at dusk to include zooplankton that migrate vertically throughout the day.

Sampling procedure and laboratory analyses

We collected whole-water samples for chemical analyses by lowering a length of 6-mm-diameter Tygon tubing to 3.5 m. An in-

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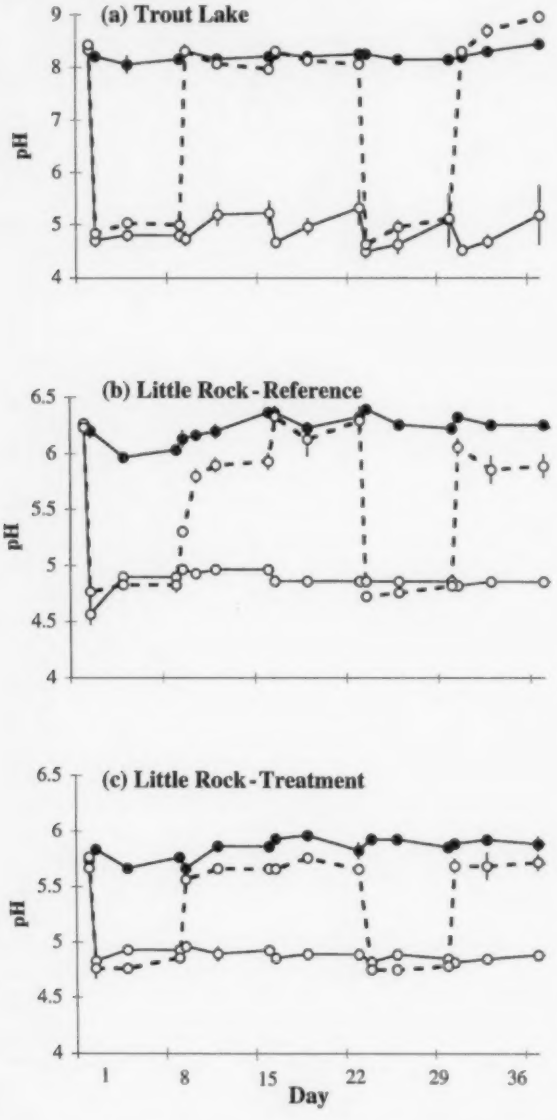
Fig. 1. Mean and standard error of pH throughout the course of the experiment in (*a*) Trout Lake, (*b*) Little Rock - Reference, and (*c*) Little Rock - Treatment. The solid line with solid circles denotes the control treatment, the solid line with open circles denotes the press treatment, and the broken line with open circles denotes the pulse treatment.



tegrated water sample was trapped in the tube by raising the tube from the bottom. Samples for DIC and dissolved organic carbon (DOC) analysis were collected three times during the experiment and analyzed using an O/I Corporation total organic carbon analyzer. Temperature, dissolved oxygen, and Secchi depth were measured weekly.

We collected phytoplankton from each bag twice per week using the tube sampler described above. We preserved phytoplankton with glutaraldehyde (1% final concentration) and enumerated the samples using the Utermohl method. Preserved 25-mL subsamples were gravity settled for at least 22 h and counted on an inverted microscope at 400× magnification. At least 25 fields or 200 cells were counted per sample. We calculated biovolume using geometrical formulas according to the shape of each alga (Wetzel and Likens 1991). Most phytoplankton were identified to genus. We were unable to identify the species of *Mougeotia* in our experiment because zygospores were not present. Observations of cell dimensions and chloroplast shape suggest that Trout Lake may have contained two species of *Mougeotia*. The dominant form in Trout Lake had similar dimensions and chloroplast shape to the one species present in Little Rock - Reference and Little Rock - Treatment.

Zooplankton were collected from each bag once a week at 0.5, 1.5, 2.5, and 3.5 m below the surface using a 12-L Schindler–Patalas trap with an 80-μm-mesh net. All four samples were combined and preserved with cold sucrose-buffered formalin (5% final concentration). We counted and measured zooplankton length at 50× magnification on a dissecting microscope. Cladocerans and adult copepods were identified to species. Copepod copepodites were combined into two categories: calanoid or cyclopoid. We calculated biomass using length–weight regressions after McCauley (1984), with the exception of *Holopedium gibberum* whose biomass without the gelatinous sheath was estimated using the equation in Peters and Downing (1984).

Analyses

We used repeated measures analysis of variance (ANOVA) to assess time effects, treatment effects, and time × treatment interactions on the biomass/biovolume of individual taxa and functional groups (Gurevitch and Chester 1986). We believe that one of the Trout Lake control bags was invaded by fish because large-bodied zooplankton disappeared from the mesocosm during a 3-day period whereas small-bodied zooplankton tracked the pattern of the other controls. Therefore, we excluded this bag from all analyses. We applied a square root transformation to normalize the data and homogenize variances. The symmetry of the covariance matrix did not meet the assumptions of repeated measures ANOVA. However, the Huynh–Feldt conditions were satisfied, and therefore, we used the Huynh–Feldt corrected p values (Potvin et al. 1990).

Autoregressive models

We used a first-order autoregressive model to identify the potential mechanisms responsible for generating the patterns of *Mougeotia* biovolume among systems (Box et al. 1994; Ives et al. 1999; Klug et al. 2000). The advantage of using this type of model is that we were able to look at the relative importance of a number of factors simultaneously. We fit an autoregressive model of *Mougeotia* population growth rate between successive samples where the predictors were the biovolume of the other dominant taxa in the phytoplankton community, zooplankton biomass, pH, temperature, DIC, DOC, and categorical variables for each lake. Data from all bags were combined in the analysis. The model was of the form

$$(1) \qquad x(t+1) = a + bx(t) + \sum_{k=1}^{m} c_k u_k(t) + \varepsilon(t)$$

where $x(t)$ is the biovolume of *Mougeotia* in the sample at time t, b is the effect of *Mougeotia* at time t on the change in *Mougeotia* biovolume, $u_k(t)$ is the value of a covariate in the model (such as pH or temperature), a is a constant, c_k is the effect of $u_k(t)$ on the change in *Mougeotia* biovolume, and $\varepsilon(t)$ is unexplained variability. Variables that were collected at lower frequency than phytoplankton were linearly interpolated. Data were $\log(x + c)$ transformed before analysis where c is a constant. We chose the value of c to normalize the error terms $\varepsilon(t)$. In addition, data were standardized by dividing by the standard deviation calculated across all lakes and dates. With this standardization, model coefficients represented an "aggregate" effect that combines per capita effects and variation in densities. This makes the magnitude of the

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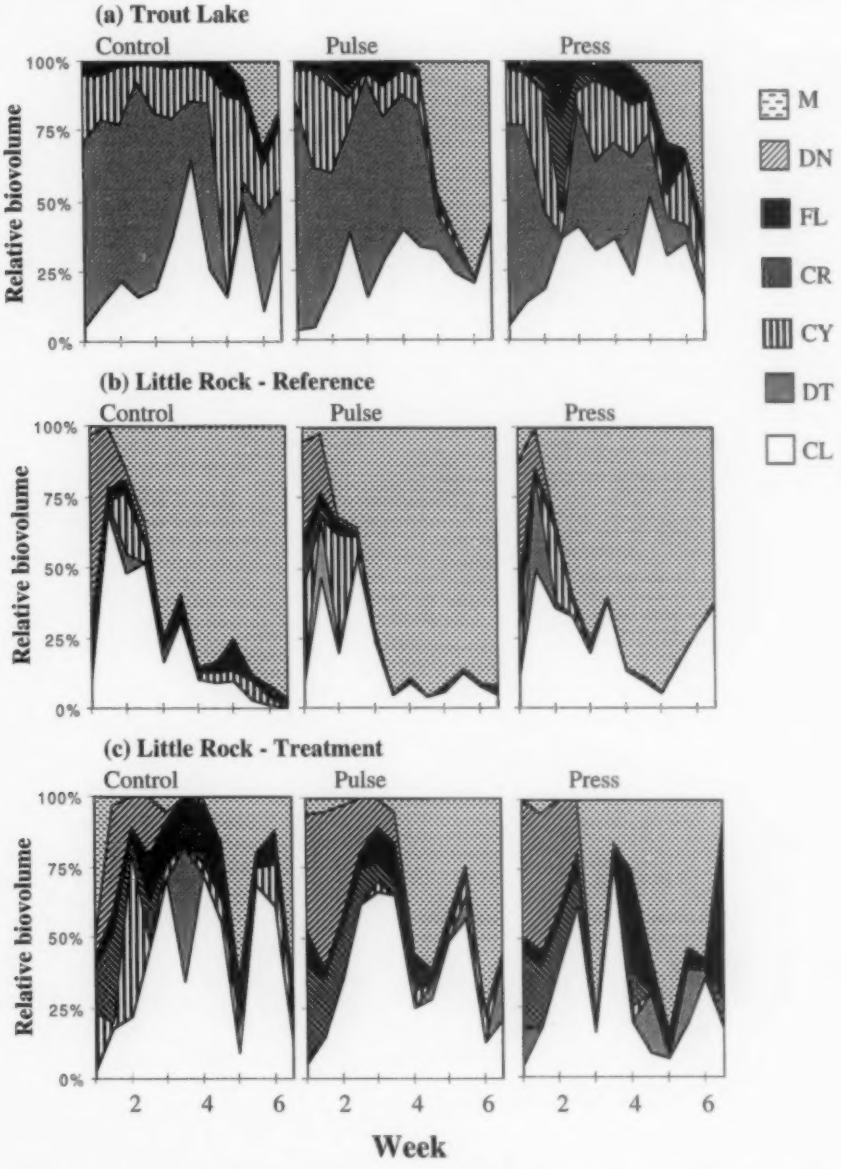
Fig. 2. Relative biovolume of *Mougeotia* (M), dinoflagellates (DN), small flagellates (FL), chrysophytes (CR), cyanobacteria (CY), diatoms (DT), and chlorophytes (CL) in the control, pulse, and press treatments over time in (*a*) Trout Lake, (*b*) Little Rock - Reference, and (*c*) Little Rock - Treatment.



coefficients c_k comparable in terms of the total impact of covariates $u_k(t)$ on changes in *Mougeotia* biovolume (Neter et al. 1989).

We fit the autoregressive model using least-squares techniques. The best-fitting subset model was chosen as that with the lowest Bayesian information criterion (Box et al. 1994). The Bayesian information criterion is a measure of model fit that includes a penalty for the number of parameters. We used an algorithm to identify the parameters in the best-fitting model through an exhaustive search of all possible models. Covariates in the model included pH, temperature, DIC, DOC, small herbivorous zooplankton, large herbivorous zooplankton, other dominant phytoplankton taxonomic groups (other chlorophytes, diatoms, and dinoflagellates), and categorical variables for the different lakes. The categorical variables were used to identify interlake differences in *Mougeotia* growth rate that may be caused by variables that we did not measure (e.g., nutrients). We used a cutoff value of 0.5 mm to separate small and large herbivorous zooplankton.

Results

Biotic variables

As anticipated, initial phytoplankton community composition was different among lakes (Fig. 2). Trout Lake was dominated by diatoms whereas Little Rock - Reference and

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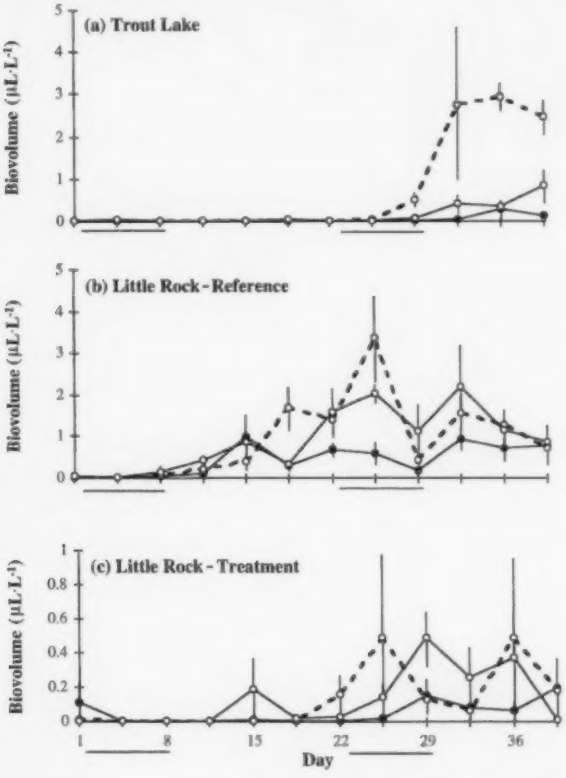
Fig. 3. Mean and standard error of *Mougeotia* biovolume in (*a*) Trout Lake, (*b*) Little Rock - Reference, and (*c*) Little Rock - Treatment over time. Note the difference in scale on the *y*-axis. Lines under the *x*-axis denote the time periods of the pulse treatment when the pH was low. The solid line with solid circles denotes the control treatment, the solid line with open circles denotes the press treatment, and the broken line with open circles denotes the pulse treatment.



Little Rock - Treatment were dominated by dinoflagellates. In Little Rock - Treatment, *Mougeotia* biovolume appears to be initially higher in the control treatment than in the pulse or the press treatment. This represents one control bag that happened to contain more *Mougeotia* at the start of the experiment. The dominance of diatoms and dinoflagellates decreased over time in all treatments. Throughout the course of the experiment, diatoms were rare in both Little Rock - Reference and Little Rock - Treatment whereas dinoflagellates were rare in Trout Lake (Fig. 2).

Mougeotia increased significantly with time in all treatments in all three lakes, although there were differences in the magnitude of increase among treatments and lakes. On average, Little Rock - Reference had the highest biovolume of *Mougeotia*, and Little Rock - Treatment had the lowest biovolume (Fig. 3). Within lakes, *Mougeotia* biovolume tended to be higher in acidified treatments than in the control treatment, although this pattern was only statistically significant (after Bonferroni correction) in Trout Lake (Table 2).

Zooplankton communities changed throughout the course of the experiment (Fischer 1997). In Trout Lake, *Daphnia galeata mendotae* was the dominant large herbivore in the control treatment whereas *Holopedium gibberum* was the dominant large herbivore in the acidified treatments. Large herbivore biomass increased throughout the experiment in Trout Lake, although biomass was significantly higher in the control treatment than in either of the acidified treatments (Fig. 4). In contrast, large herbivore biomass was not significantly different (after Bonferroni correction) among treatments in Little Rock - Reference or Little Rock - Treatment (Table 2; Fig. 4). Large herbivore dynamics in Little Rock - Reference and Little Rock - Treatment were primarily driven by *H. gibberum* and *Sida crystallina*. In all three lakes, biomass of small herbivorous zooplankton (*Bosmina longirostris* and *Chydorus sphericus*) tended to be higher in the acidified treatments than in the control treatment (Fischer 1997).

Abiotic variables

pH varied among treatments in accordance with our experimental design (see Methods and Fig. 1). DIC was highest in Trout Lake and lowest in Little Rock - Treatment (Fig. 5). In general, changes in pH should be accompanied by changes in DIC (Stumm and Morgan 1996). However, in our experiment, the sudden changes in pH that we imposed in the pulse treatments were not always followed by changes in DIC concentration. Days 18 and 32 were periods when pH values in the pulse treatments were similar to levels in the control treatments. In Trout Lake and Little Rock - Reference, however, DIC concentration was much lower in the pulse treatments relative to the control treatments. In Trout Lake, this pattern led to conditions of high pH but DIC concentrations much lower than would be expected (Table 3).

Changes in light may affect phytoplankton biovolume and community composition. Light availability may have changed in our experiment due to pH-induced changes in DOC. DOC was generally low in all three lakes (~3.25 $mg \cdot L^{-1}$) and decreased by approximately 0.5 $mg \cdot L^{-1}$ in the acidified treatments (data not shown). We made no measure of actual light; however, Secchi depth was usually deeper than the depth of the mesocosms (>4 m expect for two of the pulse treatment bags (Secchi = 3.5 and 3.75 m) in Trout Lake on 24 July). DOC data were included in the autoregressive model to account for differences in light availability.

Autoregressive models

The best-fitting model is presented in Fig. 6. Model results suggest that both biotic and abiotic factors influenced the growth of *Mougeotia* in the experiment (Fig. 6). Acidity, DIC, and dinoflagellates had negative effects on *Mougeotia* growth rate. In addition, one of the lake categorical variables was in the best-fitting model, suggesting that *Mougeotia* growth rate was lower in Little Rock - Treatment than in Trout Lake and Little Rock - Reference. Predictor variables varied in the magnitude of their effects (Fig. 6). DIC had the largest effect on *Mougeotia* growth rate, and acidity had the smallest effect.

We calculated model fit in two ways: first, by comparing predicted biovolume at time t with observed biovolume at time t and, second, by comparing predicted change in biovolume from time t to time $t + 1$ with observed changes in

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Table 2. Results of univariate repeated measures ANOVA for response to experimental treatment.

Response variable	Time	Treatment	Contrasts	Time × treatment	Contrasts
Trout Lake					
Mougeotia	**0.0001**	**0.0033**	C PR PU	**0.0028**	C PR PU
pH	**0.0001**	**0.0001**	C PU PR	**0.0001**	C PU PR
Temperature	**0.0001**	ns		ns	
DIC	**0.0001**	**0.0001**	C PU PR	**0.0001**	C PU PR
DOC	0.0607	**0.003**	C PU PR	0.0189	C PU PR
Large herbivorous zooplankton	**0.0001**	**0.0001**	C PU PR	**0.0001**	C PU PR
Small herbivorous zooplankton	**0.0001**	ns		**0.0018**	C PU PR
Chlorophytes	0.088	ns		0.055	
Chrysophytes	**0.001**	ns		0.033	C PU PR
Cyanobacteria	0.011	0.034	C PR PU	ns	
Diatoms	**0.0001**	0.092		**0.0005**	C PU PR
Dinoflagellates	ns	ns		ns	
Flagellates	ns	ns		ns	
Little Rock - Reference					
Mougeotia	**0.0001**	0.0348		ns	
pH	**0.0001**	**0.0001**	C PU PR	**0.0001**	C PU PR
Temperature	**0.0001**	ns		ns	
DIC	**0.0001**	**0.0002**	C PU PR	**0.0001**	C PU PR
DOC	**0.0001**	0.0153	C PR PU	0.0120	C PU PR
Large herbivorous zooplankton	**0.0001**	0.0496		0.0474	C PR PU
Small herbivorous zooplankton	**0.0013**	ns		ns	
Chlorophytes	**0.0006**	0.0219	C PU PR	0.0268	C PU PR
Chrysophytes	**0.0001**	0.0065	C PU PR	ns	
Cyanobacteria	**0.0001**	ns		0.0234	C PU PR
Diatoms	**0.0017**	ns		ns	
Dinoflagellates	**0.0001**	ns		ns	
Flagellates	**0.0001**	0.0081	C PU PR	**0.0037**	C PU PR
Little Rock - Treatment					
Mougeotia	**0.0033**	ns		ns	
pH	**0.0001**	**0.0001**	C PU PR	**0.0001**	C PU PR
Temperature	**0.0001**	ns		ns	
DIC	**0.0001**	ns		ns	
DOC	**0.0001**	0.0274	PU C PR	0.0135	PU C PR
Large herbivorous zooplankton	**0.0001**	ns		ns	
Small herbivorous zooplankton	**0.0005**	0.0170	C PU PR	ns	
Chlorophytes	**0.0027**	ns		ns	
Chrysophytes	**0.0001**	**0.0035**	C PU PR	0.0355	C PR PU
Cyanobacteria	0.074	**0.0021**	C PU PR	ns	
Diatoms	ns	ns		ns	
Dinoflagellates	**0.0001**	ns		0.0282	C PU PR
Flagellates	**0.0056**	ns		ns	

Note: All p values <0.10 have been reported (ns signifies $p > 0.10$). Bold p values were significant at $\alpha < 0.05$ after sequential Bonferroni correction for the number of variables tested per lake. Lines connecting letters indicate treatments that were not significantly different after Bonferroni correction for number of treatments ($\alpha < 0.05$). C, control treatment; PU, pulse treatment; PR, press treatment.

biovolume from time t to time $t + 1$. The R^2 values for static biovolume and change in biovolume are 0.65 and 0.36, respectively. We used normal probability plots and plots of the residuals versus observed values to test the assumptions of the autoregressive models.

We used the model results to generate hypotheses about mechanisms causing the differences in *Mougeotia* biovolume across lakes and treatments. Maximum biovolume of *Mougeotia* was observed in Trout Lake in the pulse treatment when pH was high (acidity was low) and DIC was low (Fig. 3). In Trout Lake, we hypothesize that the direct negative effect of acidity prevented a *Mougeotia* bloom in the press treatment, and high DIC constrained *Mougeotia* growth in the control treatment. Treatment effects on the predictor variables were weaker (either not significant or of lower magnitude) in Little Rock - Reference and Little Rock - Treatment relative to Trout Lake. This may help explain the lack of treatment effects on *Mougeotia* biovolume. *Mou-*

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Fig. 4. Mean and standard error of large herbivorous zooplankton biomass in (*a*) Trout Lake, (*b*) Little Rock - Reference, and (*c*) Little Rock - Treatment over time. Note the difference in scale on the *y*-axis. The solid line with solid circles denotes the control treatment, the solid line with open circles denotes the press treatment, and the broken line with open circles denotes the pulse treatment.

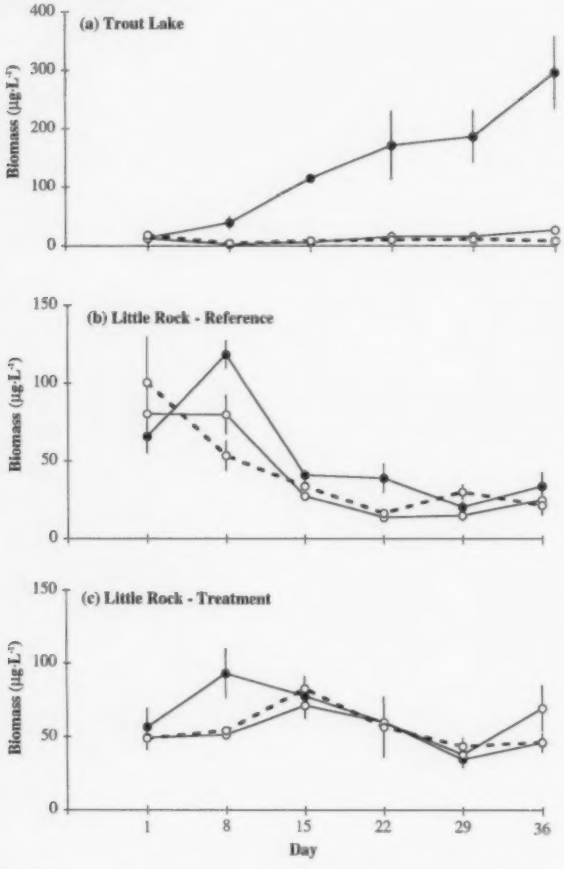
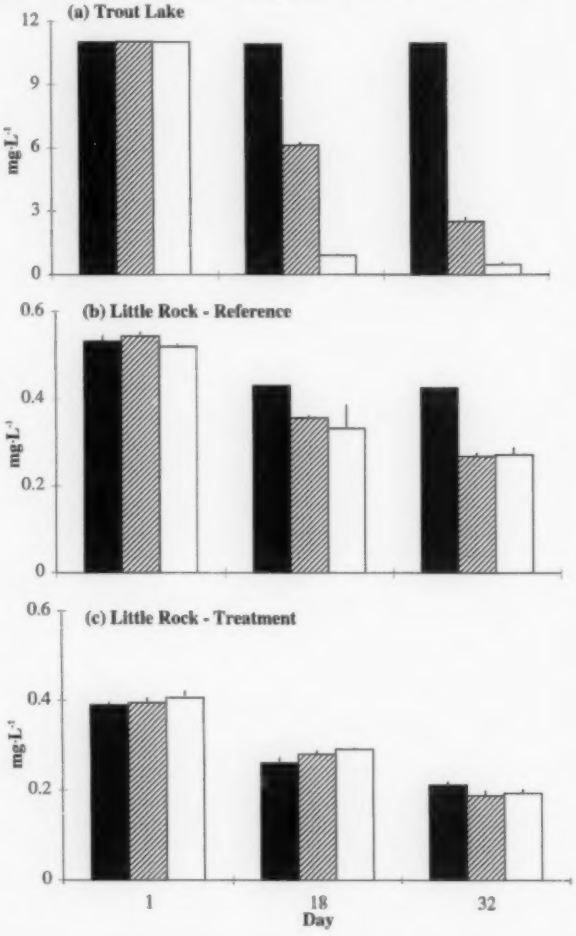


Fig. 5. Mean and standard error of DIC concentration in (*a*) Trout Lake, (*b*) Little Rock - Reference, and (*c*) Little Rock - Treatment. Note the difference in scale on the *y*-axis. The solid bars denote the control treatment, the hatched bars denote the pulse treatment, and the open bars denote the press treatment.



geotia biovolume increased over time in both Little Rock - Reference and Little Rock - Treatment, corresponding to decreases in DIC and dinoflagellates. At the start of the experiment, total phosphorus and total nitrogen concentrations were much lower in Little Rock - Treatment than in Trout Lake and Little Rock - Reference, which may explain why, on average, *Mougeotia* biovolume was lowest in Little Rock - Treatment (Table 1).

A caveat is needed when interpreting the autoregressive model. Autoregressive models rely on correlated changes in variables. The best-fitting model gives the best post hoc description of changes in *Mougeotia* biovolume, but as with all models based on correlation, it does not constitute a test of interactions.

Discussion

As in previous studies of phytoplankton responses to acidification (Findlay and Kasian 1990; Olaveson and Nalewajko 1994), we observed dramatic changes in species composition following experimental acidification. Despite differences in original phytoplankton community composition among lakes, the most striking result was an increase in the filamentous green alga *Mougeotia* in all three systems (Figs. 2 and 3). Differences in the magnitude of increase among lakes and treatments allowed us to explore the biotic and abiotic factors responsible for regulating *Mougeotia* growth rate at both high and low pH. Our results suggest that *Mougeotia* growth rate was mostly affected by pH and DIC. Competition with other algae played a lesser role.

Model results and patterns from Trout Lake suggest that acidity has a negative effect on *Mougeotia* growth. This result is counterintuitive, given the abundance of surveys and experiments that show proliferation of *Mougeotia* at low pH (e.g., Turner et al. 1991; Webster et al. 1992; Fairchild and Sherman 1993). However, none of these studies documented a direct physiological effect of acidity on *Mougeotia*. Gra-

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Table 3. DIC and pH concentrations in Trout Lake.

Treatment	Day	DIC	pH
Control	1	11.0±0.004	8.4±0
	18	11.0±0.03	8.4±0.06
	32	10.9±0.05	8.3±0
Pulse	1	11.0±0.03	8.4±0.06
	18	6.1±0.32	8.1±0.15
	32	2.5±0.63	8.7±0.20
Press	1	11.0±0.006	8.3±0.06
	18	0.91±0.12	5.0±0.25
	32	0.47±0.29	4.7±0.20

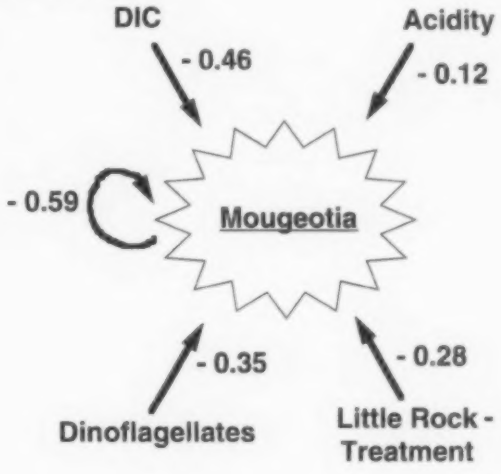
Note: Values are treatment means ± 1 SD. Note that DIC concentrations in the pulse treatment on days 18 and 32 are less than would be expected at high pH.

ham et al. (1996) showed that when grown alone, gross photosynthesis by *Mougeotia* was highest at pH = 8.0 in *Mougeotia* isolated from an acidified lake (Little Rock - Treatment). Direct effects of acidity are difficult to discern from effects of other variables that change with pH (e.g., DIC and metals). Our experiment provided a unique test because DIC and pH were not as tightly correlated as would be expected due to the rapid changes in pH imposed in the pulse treatment (Table 3). This allowed us to begin to tease apart the effects of DIC and acidity.

In general, DIC concentration was negatively related to *Mougeotia* growth rate. This is in contrast with other studies in which filamentous green algae were positively related to DIC (Turner et al. 1994, 1995*b*). These studies suggest that benthic algae are DIC limited at low pH; therefore, growth increases at higher DIC (Turner et al. 1987; Howell et al. 1990). The *Mougeotia* in our experiment were planktonic and may have been less DIC limited because they have a thinner boundary layer than benthic algae (Turner et al. 1991). The uptake rate of dissolved substances is inversely related to boundary layer thickness (Riber and Wetzel 1987). Additionally, limiting DIC concentrations may be the only environment in which *Mougeotia* can outcompete other algae, thereby leading to the lower growth rates at high DIC concentration. Although *Mougeotia* growth was usually higher when DIC was low, on average, *Mougeotia* biovolume was lowest in Little Rock - Treatment, the lake with the lowest DIC concentrations. The categorical lake variable suggests that *Mougeotia* growth rate in Little Rock - Treatment was lower due to factors not included in the model. This may represent the lower phosphorus and nitrogen concentrations in Little Rock - Treatment. These effects presumably overshadowed the benefit of low DIC in Little Rock - Treatment.

Herbivory and competition with other algae are factors that have been hypothesized to limit *Mougeotia* growth (Turner et al. 1991; Graham et al. 1996). Our results show no effects of zooplankton grazers on *Mougeotia* growth; however, fish, crayfish, tadpoles, and macroinvertebrates may be important grazers on filamentous green algae (e.g., Power and Matthews 1983; France and Welbourn 1992; Graham and Vinebrooke 1998) and were absent from this experiment. Model results suggest that, when present, dinoflagellates had a negative effect on *Mougeotia* growth presumably due to competition. Dinoflagellates were initially one of the dominant phytoplankton groups in Little Rock - Reference and Little Rock - Treatment. Dinoflagellates and *Mougeotia* are relatively slow-growing taxa (Sandgren 1988) and may have been competing for nutrients such as nitrogen and phosphorus.

Fig. 6. Coefficients in the best-fitting autoregressive model. Each arrow represents a term in the model. Pluses and minuses indicate positive and negative effects. The negative term for Little Rock - Treatment indicates that, on average, *Mougeotia* growth rate was lower in Little Rock - Treatment than in Trout Lake and Little Rock - Reference due to unidentified factors not included in the model.



Mougeotia is primarily a benthic species. However, *Mougeotia* can be planktonic, and all of our observations were of planktonic *Mougeotia*. The densities reached in some of the treatments were greater than could be expected simply by sloughing off the walls (especially since there was very little visible wall growth). Recent work by Linda Graham and her colleagues has confirmed that *Mougeotia* has a dispersed, planktonic growth form, as we saw in our experiment. They found that *Mougeotia* isolated from Little Rock - Treatment was well dispersed when grown at pH = 8 and was aggregated into discrete, floating metaphytic-type clumps when grown at pH = 5 (L. Graham, personal communication).

Although in our experiment, we observed no obvious qualitative differences in growth on the mesocosm walls among treatments and lakes, the mesocosm walls probably provided favorable attachment sites for *Mougeotia*, which broke off and grew in the plankton as well. Care was taken in stirring and sampling to avoid scraping the plastic walls. Although *Mougeotia* was most certainly attached to the mesocosm walls, our conclusions are based on comparisons among mesocosms, all of which had walls; therefore, the potential for bias is low. Nonetheless, quantification of total *Mougeotia* within each mesocosm is impossible without data on wall growth, and future studies should include sampling of epiplastic algae in their design.

Several studies have highlighted the importance of abiotic factors in regulating periphyton communities at low pH (Turner et al. 1994; Vinebrooke 1996; Graham and Vinebrooke 1998). Our results suggest that planktonic *Mougeotia*

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was primarily regulated by abiotic factors at both low and high pH. Biotic factors such as competition with other algae played a relatively minor role in regulating *Mougeotia* growth. Rather, pH and abiotic factors associated with changes in pH (e.g., DIC) were the most important predictors of *Mougeotia* growth. Although filamentous green algae in general are stimulated by increases in DIC concentration (Turner et al. 1987; Fairchild and Sherman 1992), *Mougeotia* appears to be favored only at very low DIC concentrations (Fairchild and Sherman 1993; this study). We suggest that this benefit of low DIC concentration is enough to compensate for the negative effect of acidity. Studies designed explicitly to test this hypothesis will further enhance our understanding of this important alga.

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References

Box, G.E.P., Jenkins, G.M., and Reinsel, G.C. 1994. Time series analysis. Prentice Hall, Englewood Cliffs, N.J.

Brezonik, P.L., Eaton, J.G., Frost, T.M., Garrison, P.J., Kratz, T.K., Mach, C.E., McCormick, J.H., Perry, J.A., Rose, W.A., Sampson, C.J., Shelley, B.C.L., Swenson, W.A., and Webster, K.E. 1993. Experimental acidification of Little Rock Lake, Wisconsin: chemical and biological changes over the pH range 6.1 to 4.7. Can. J. Fish. Aquat. Sci. **50**: 1101–1121.

Fairchild, G.W., and Sherman, J.W. 1992. Linkage between epilithic algal growth and water column nutrients in softwater lakes. Can. J. Fish. Aquat. Sci. **49**: 1641–1649.

Fairchild, G.W., and Sherman, J.W. 1993. Algal periphyton response to acidity and nutrients in softwater lakes: lake comparison vs. nutrient enrichment approaches. J. North Am. Benthol. Soc. **12**: 157–167.

Findlay, D.L., and Kasian, S.E.M. 1990. Phytoplankton communities of lakes experimentally acidified with sulfuric and nitric acids. Can. J. Fish. Aquat. Sci. **47**: 1378–1386.

Fischer, J.M. 1997. Zooplankton community responses to acidification: the role of evolution and compensatory dynamics. Ph.D. thesis, University of Wisconsin-Madison, Madison, Wis.

France, R.L., and Welbourn, P.M. 1992. Influence of lake pH and macrograzers on the distribution and abundance of nuisance metaphytic algae in Ontario, Canada. Can. J. Fish. Aquat. Sci. **49**: 185–195.

Graham, J.M., Arancibia-Avila, P., and Graham, L.E. 1996. Effects of pH and selected metals on growth of the filamentous green algae *Mougeotia* under acidic conditions. Limnol. Oceanogr. **41**: 263–270.

Graham, M.D., and Vinebrooke, R.D. 1998. Trade-offs between herbivore resistance and competitiveness in periphyton of acidified lakes. Can. J. Fish. Aquat. Sci. **55**: 806–814.

Gurevitch, J., and Chester, S.T., Jr. 1986. Analysis of repeated measures experiments. Ecology, **67**: 251–255.

Howell, E.T., Turner, M.A., France, R.L., Jackson, M.B., and Stokes, P.M. 1990. Comparison of zygnematacean (*Chlorophyta*) algae in the metaphyton of two acidic lakes. Can. J. Fish. Aquat. Sci. **47**: 1085–1092.

Ives, A.R., Carpenter, S.R., and Dennis, B. 1999. Community interaction webs and zooplankton responses to planktivory manipulations. Ecology, **80**: 1405–1421.

Jackson, M.B., Vandermeer, E.M., Lester, N., Booth, J.A., Molot, L., and Gray, I.M. 1990. Effects of neutralization and early reacidification on filamentous algae and macrophytes in Bowland Lake. Can. J. Fish. Aquat. Sci. **47**: 432–439.

Klug, J.L., Fischer, J.M., Ives, A.R., and Frost, T.M. 2000. Compensatory dynamics in planktonic community responses to pH perturbations. Ecology, **81**: 387–398.

McCauley, E. 1984. The estimation of the abundance and biomass of zooplankton in samples. *In* A manual on methods for the assessment of secondary productivity in fresh waters. *Edited by* F.H. Rigler and J.A. Downing. Blackwell Scientific Publications, Oxford, U.K. pp. 228–265.

Müller, P. 1980. Effects of artificial acidification on the growth of periphyton. Can. J. Fish. Aquat. Sci. **37**: 355–363.

Neter, J., Wasserman, W., and Kutner, M.H. 1989. Applied linear regression models. Irwin, Homewood, Ill.

Okland, J. 1980. Environment and snails (*Gastropoda*): studies of 1000 lakes in Norway. *In* Ecological Impact of Acid Precipitation, Proceedings of an International Conference, Sandefjord, Norway, March 11–14, 1980. *Edited by* A.T. Drablos. SNSF Project, Oslo, Norway. pp. 322–323.

Olaveson, M.M., and Nalewajko, C. 1994. Acid and freshwater algae. *In* Algae and water pollution. Advances in Limnology. Vol. 42. *Edited by* L.C. Rai, J.P. Gaur, and C.J. Soeder. Schweigerbart, Stuttgart, Germany. pp. 99–123.

Peters, R.H., and Downing, J.A. 1984. Empirical analysis of zooplankton filtering and feeding rates. Limnol. Oceanogr. **29**: 763–784.

Potvin, C., Lechowicz, M.J., and Tardif, S. 1990. The statistical analysis of ecophysiological response curves obtained from experiments involving repeated measures. Ecology, **71**: 1389–1400.

Power, M.E., and Matthews, W.J. 1983. Algae-grazing minnow (*Campostoma anomalum*), piscivorous bass (*Micropterus* spp.), and the distribution of attached algae in a small prairie-margin stream. Oecologia, **60**: 328–332.

Riber, H.H., and Wetzel, R.G. 1987. Boundary-layer and internal diffusion effects on phosphorus fluxes in lake periphyton. Limnol. Oceanogr. **32**: 1181–1194.

Sandgren, C.D. 1988. Growth and reproductive strategies of freshwater phytoplankton. Cambridge University Press, Cambridge, U.K.

Schindler, D.W., Mills, K.H., Malley, D.F., Findlay, D.L., Shearer, J.A., Davies, I.J., Turner, M.A., Linsey, G.A., and Cruikshank, D.R. 1985. Long-term ecosystem stress: the effects of years of experimental acidification on a small lake. Science (Washington, D.C.), **228**: 1395–1401.

SPR Assoc. Inc. 1986. Estimation of the presence and impact of filamentous and odour-producing algae: a survey of cottagers on 214 Ontario recreational lakes. (Available from the Ontario Ministry of the Environment, Toronto, Ont.)

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Stumm, W., and Morgan, J.J. 1996. Aquatic chemistry: chemical equilibria and rates in natural waters. John Wiley & Sons, Inc., New York.

Turner, M.A., Jackson, M.B., Findlay, D.L., Graham, R.W., DeBruyn, E.R., and Vandermeer, E.M. 1987. Early responses of periphyton to experimental lake acidification. Can. J. Fish. Aquat. Sci. **44**(Suppl. 1): 135–149.

Turner, M.A., Howell, E.T., Summerby, M., Hesslein, R.H., Findlay, D.L., and Jackson, M.B. 1991. Changes in epilithon and epiphyton associated with experimental acidification of a lake to pH 5. Limnol. Oceanogr. **36**: 1390–1405.

Turner, M.A., Howell, E.T., Robinson, G.G.C., Campbell, P., Hecky, R.E., and Schindler, E.U. 1994. Roles of nutrients in controlling growth of epilithon in oligotrophic lakes of low alkalinity. Can. J. Fish. Aquat. Sci. **51**: 2784–2793.

Turner, M.A., Schindler, D.W., Findlay, D.L., Jackson, M.B., and Robinson, G.G.C. 1995*a*. Disruption of littoral algal associations by experimental lake acidification. Can. J. Fish. Aquat. Sci. **52**: 2238–2250.

Turner, M.A., Howell, E.T., Robinson, G.G.C., Brewster, J.F., Sigurdson, L.J., and Findlay, D.L. 1995*b*. Growth characteristics of bloom-forming filamentous green algae in the littoral zone of an experimentally acidified lake. Can. J. Fish. Aquat. Sci. **52**: 2251–2263.

Vinebrooke, R.D. 1996. Abiotic and biotic regulation of periphyton in recovering acidified lakes. J. North Am. Benthol. Soc. **41**: 1035–1040.

Webster, K.E., Frost, T.M., Watras, C.J., Swenson, W.A., Gonzalez, M., and Garrison, P.J. 1992. Complex biological responses to the experimental acidification of Little Rock Lake, Wisconsin, U.S.A. Environ. Pollut. **78**: 73–78.

Wetzel, R.G., and Likens, G.E. 1991. Limnological analyses. Springer-Verlag, New York.

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Role of satiation in the functional response of a piscivore, largemouth bass (*Micropterus salmoides*)

Timothy E. Essington, James R. Hodgson, and James F. Kitchell

Abstract: We evaluated whether satiation regulates the predation rates of a piscivore, largemouth bass (*Micropterus salmoides*), in natural settings. A functional response model indicated that predation rates can be reduced by satiation when mean prey density is high or when prey encounters are highly patchy. We then used bioenergetics modeling to estimate the predation rates of individual bass in four lakes during a 16-year period and used stomach content mass in diet samples to evaluate the variability in daily predation rates. Predation rates, expressed as the proportion (p) of the maximum daily consumption rate, were low (mode = 0.3, mean = 0.4). Stomach fullness (s), expressed as the proportion of the stomach fullness associated with the maximum sustainable consumption rate, was highly variable, and 13% of all bass diets had $s > 1$, indicating that bass could opportunistically forage at rates exceeding their maximum sustainable rate. The low predation rates and the ability to consume prey at rates exceeding the maximum sustainable rate make it unlikely that satiation was an important constraint on bass predation rates. Thus, satiation effects widely represented in modeling studies may be a rare component in piscivore–prey interactions, while prey behavior may be a more important component governing predation rates.

Résumé : Nous avons évalué si la satiété régulait les taux de prédation d'un piscivore, l'achigan à grande bouche (*Micropterus salmoides*), en milieu naturel. Un modèle de réponse fonctionnelle a indiqué que les taux de prédation peuvent être réduits par la satiété quand la densité moyenne de proies est élevée ou quand les rencontres de proies sont très intermittentes. Nous avons ensuite utilisé la modélisation bioénergétique pour estimer les taux de prédation individuels d'achigans dans quatre lacs durant 16 ans et mesuré la masse d'échantillons de contenus stomacaux pour évaluer la variabilité des taux quotidiens de prédation. Les taux de prédation, exprimés comme la proportion (p) des prises par rapport au taux maximum de consommation quotidienne, étaient faibles (mode = 0,3, moyenne = 0,4). Le degré de remplissage de l'estomac (s), exprimé comme la proportion du contenu stomacal par rapport au degré de remplissage de l'estomac associé au taux de consommation soutenable maximum, était très variable, et 13% de tous les contenus stomacaux donnaient des valeurs de s supérieures à 1, ce qui indique que les achigans peuvent de façon opportuniste consommer des proies à des taux excédant leur taux soutenable maximum. Les faibles taux de prédation et la capacité des achigans de consommer des proies à des taux excédant leur taux soutenable maximum font qu'il est peu probable que la satiété influe de façon importante sur les taux de prédation des achigans. Ainsi, les effets liés à la satiété largement signalés dans les études de modélisation pourraient n'intervenir que rarement dans les interactions entre piscivores et proies, tandis que le comportement de ces dernières pourrait influer davantage sur les taux de prédation.

[Traduit par la Rédaction]

Introduction

The functional response describes the relationship between the density of prey and the rate at which individual predators consume them (Solomon 1949; Holling 1959). Although the exact form of this relationship varies, nearly all forms include prey density-dependent predation rates over some range of prey densities and prey density-independent predation rates at high prey densities. The shape of the functional response curve has strong effects on predator–prey dynamics because predation mortality may be either density dependent, inversely density dependent, or density independent (Murdoch and Oaten 1975; Hassell 1978). Further, nonlinear functional responses coupled with dome-shaped recruitment functions can lead to multiple stable states in predator and prey abundances (May 1977).

Predator–prey models with nonlinear functional responses often predict oscillatory or unstable population dynamics, yet data from natural systems often fail to match these predictions (e.g., Murdoch 1994). One way to resolve these discrepancies is to determine whether predation rates in natural settings are in a range where constraints associated with the density-independent portion of the curve are important. In other words, how often are predation rates on the asymptotic portion of the functional response?

This question is typically addressed by fitting a functional response curve to observed predation rates over a wide range of prey densities. However, quantifying the functional re-

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sponse in natural settings may be problematic, owing to difficulties in making realistic estimates of prey availability. Further, Holling's (1959) seminal paper described the functional response as the instantaneous predation rate of a predator at a fixed prey density. In contrast, field estimates of predation rates are typically integrated over longer time periods during which prey densities may fluctuate considerably. Most functional response curves have been fit to experimental data wherein prey abundance can be maintained at fixed densities and distributed homogeneously. However, laboratory experiments do not incorporate temporal or spatial variation in prey availability and potentially suffer from confounding scale effects (Carpenter and Kitchell 1987). The applicability of such laboratory experiments to natural settings has been questioned (Hodgson and Kitchell 1987; Mackenzie et al. 1990). These difficulties illustrate the need for an alternative approach for evaluating the functional response of predators in natural settings.

An alternative is to compare the dependent variable, predation rate, with a known maximum rate to provide a reference position on the functional response curve. This approach requires that one identify the important constraints, determine the predation rates at which these constraints become important, and develop a functional response model that does not require the assumption of fixed prey abundance. The latter is easily achieved by replacing the fixed prey density in the functional response with a probability distribution of prey densities (e.g., Murdoch and Oaten 1975; Hassell 1978). Two constraints are thought to be important in generating asymptotic functional response curves: searching time and physiological limits on ingestion rate (i.e., time to ingest and digest captured prey). For many predators, searching time is nearly impossible to estimate from field data and is inappropriate if derived from laboratory studies. Further, Breck (1993) argued that handling costs are negligible for piscivorous fishes because they eat few, large prey items per day. However, estimates of physiological constraints can be accurately derived in the laboratory (e.g., Niimi and Beamish 1974) and are less likely to vary markedly between natural and laboratory settings. Thus, physiological constraints provide a useful starting point for evaluating the functional response in natural settings.

In this study, we evaluated the role of a physiological constraint, satiation, on the functional response of a piscivore, largemouth bass (*Micropterus salmoides*). Largemouth bass is a keystone predator in many lakes, owing to its ability to extirpate small planktivorous fishes, thereby allowing large grazers such as *Daphnia* spp. to flourish (Carpenter et al. 1985, 1987; Hall and Ehlinger 1989; Mittelbach et al. 1995). However, at low densities, bass may have smaller effects on prey fish populations (Ramcharan et al. 1995). One possible explanation for this discrepancy is that at low densities, bass predation rates may be saturated, i.e., on the asymptotic portion of the functional response. This hypothsis can be tested by evaluating the predation rates of bass over a range of prey abundances and determine how commonly they fall on the asymptotic portion of their functional response. Ultimately, these analyses may provide important insights into why bass exert strong effects on fish community structure in some systems but not in others.

We compared the predation rates of bass in natural settings with laboratory-derived estimates of physiological maximum rates and used these data to infer their position on the functional response curve. Because prey densities are not constant over the growing season, we first developed a functional response model to evaluate how variability in prey encounters affects integrated measures of predation rate. We then estimated predation rates of individual largemouth bass in four lakes over a 16-year period that included a large range of bass and prey abundances. Finally, we evaluated the pattern of variability in prey captures and used these results to evaluate the extent to which bass predation rates were constrained by satiation.

Model development and analysis

Satiation has two components that operate at different temporal scales. At short scales (i.e., daily), satiation occurs when consumption rate exceeds the storage capacity of the stomach. At longer scales (i.e., weekly), satiation occurs when the rate of prey captures exceeds the time required to digest prey. We refer to the former as instantaneous satiation because this constraint is manifest in the instantaneous predation rates, and we refer to the latter as integrated satiation because this type of satiation occurs at time scales that correspond to the integrated predation rates typically measured from predators in natural settings. Instantaneous satiation most likely occurs at higher predation rates than integrated satiation because the former reflects a constraint on storage, whereas the latter reflects a constraint on digestion. As a starting point, we consider the simple case when these two phenomena occur at the same predation rate and then evaluate the case when storage capacity exceeds digestive capacity.

Our functional response model is based on the work of Breck (1993), which described the average predation rate of a population of piscivores as a function of mean prey density. We briefly describe Breck's (1993) functional response model here and then expand the model to include variability in daily prey encounters not treated by Breck (1993). Like any analytic model, the model sheds detail and complexity so that general properties can be evaluated. This model assumes that a maximum number of prey items can be eaten each day (C_{max}), predators consume a constant fraction (q) of prey that they encounter (R), and the variability in daily prey captures (qR) is described by a Poisson distribution. We used a daily temporal scale because most laboratory-derived estimates of maximum predation rates are measured and reported as daily predation rates. However, the temporal scale of this analytic model is arbitrary and only needs to coincide with the temporal scale of digestive clearance and satiation.

In the absence of satiation, the daily probability of consuming qR prey per day is

$$(1) \qquad P(C = qR) = \frac{(qX)^{qR}}{(qR)!} e^{-qX}$$

where X is the mean prey encounter rate. We refer to the product qX as the potential predation rate because it reflects the mean predation rate in the absence of satiation. When satiation effects are considered, the expected consumption rate C (prey per day), given C_{max}, is

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$$(2)\qquad E(C \mid C_{max}) = \sum_{C=0}^{C_{max}} CP(C = qR) + C_{max} \sum_{C=C_{max}+1}^{\infty} P(C = qR).$$

Equation 2 is Breck's (1993) functional response model. The right side of eq. 2 represents satiation in that predation rate equals C_{max} when $qR > C_{max}$. To facilitate comparisons between the analytic model and our bioenergetics-based consumption estimates (see Field methods), we scaled the predation rate by dividing it by C_{max}. This standardized predation rate, p, represents the proportion of the maximum ration consumed. Breck's (1993) functional response model predicts that p initially rises with increasing mean prey density (X) and asymptotes at high prey densities (Fig. 1). Thus, a functional response curve very similar to Holling's (1959) type II functional response can be generated, but with an integrated predation rate as the dependent variable.

Daily prey consumption may be more patchy than that described by a Poisson distribution. Therefore, we expanded the model to include other patterns of variability. We replaced eq. 1 with a negative binomial distribution such that in the absence of satiation, the daily probability of consuming qR prey is

$$(3)\qquad P(C = qR) = \frac{\Gamma(n + qR)}{\Gamma(n)(qR)!}\left(\frac{n}{n + qX}\right)^{n}\left(\frac{qX}{n + qX}\right)^{qR}$$

where X is again the mean prey encounter rate and qX is the potential predation rate. The variance in prey consumption is determined by the parameters n and X:

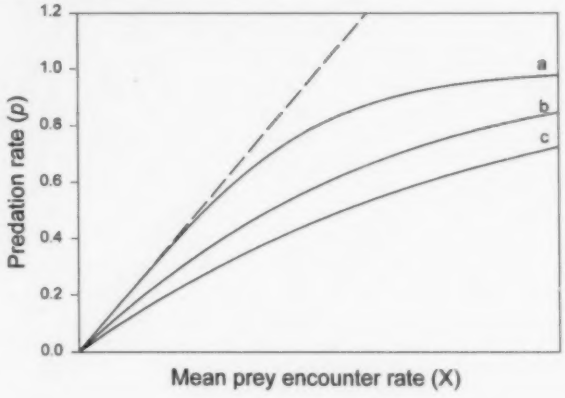
$$(4)\qquad \text{Variance} = \frac{(qX)^2}{n} + qX.$$

We adjusted n for each value of qX so that the variance to mean ratio was constant.

We examined how three patterns of variability affect the functional response curve: Poisson (variance to mean ratio = 1), patchy (negative binomial, variance to mean ratio = 5), and highly patchy (negative binomial, variance to mean ratio = 10). As prey patchiness increases, the functional response shifts downwards such that satiation begins to constrain predation rates at lower mean prey densities (Fig. 1). For the patchy scenarios (curves *b* and *c*), the functional response curve deviates from the potential predation rate when predation rates are less than $p = 0.1$ compared with $p = 0.4$ for Poisson prey encounters (curve *a*). When prey encounters are highly patchy, predation rate exceeds $p > 0.9$ only when the potential predation rate exceeds C_{max} by 3.5-fold.

Patchiness affects the functional response because of the assumptions that satiation is a threshold phenomenon and that instantaneous and integrated satiation occur at the same predation rate. When prey are highly patchy, predators encounter either few prey ($qR \ll C_{max}$) or many prey ($qR \gg C_{max}$) on a given day, and predators only consume C_{max} prey when $qR > C_{max}$. Thus, given the same mean encounter rate, predators are more likely to forego foraging opportuntities owing to satiation when prey are highly patchy than when prey encounters are described by a Poisson process. However, the effect of patchiness on the functional response can be mitigated if instantaneous satiation occurs at greater predation rates than integrated satiation. When this is true, then predators can consume prey at rates greater than C_{max} on days when $qR > C_{max}$, provided that the average predation rate is less than C_{max}. In other words, satiation may have limited effects on the functional response if predators can opportunistically consume prey when encountered at high rates.

Fig. 1. Effect of mean prey encounter rate and variability in encounter rate on predation rate. The broken line is the "potential predation rate" (qX), which indicates the predation rate in the absence of satiation. Curve *a* indicates Poisson (variance to mean ratio = 1) prey encounters, curve *b* indicates patchy (negative binomial; variance to mean ratio = 5) prey encounters, and curve *c* indicates highly patchy (negative binomial; variance to mean ratio = 10) prey encounters.



Field methods

Study site

Empirical estimates of mean summer predation rates and variability in daily predation rates were derived from field studies between 1984 and 1996 in lakes where predator and prey abundances varied and were strongly contrasted. Largemouth bass were sampled from four small kettle lakes (Paul Lake, West Long Lake, Tuesday Lake, and Peter Lake) at the University of Notre Dame Environmental Research Center located near Land O'Lakes, Wisconsin. Bass in Paul Lake were sampled from 1984 to 1996, and bass in West Long Lake were sampled from 1991 to 1996. Bass were introduced into Tuesday Lake in the spring of 1985 and remained there until removal in the fall of 1986. Bass were introduced into Peter Lake and sampled in midsummer 1996. Prior to introduction, fish communities in Tuesday and Peter lakes consisted entirely of small-bodied species (golden shiner (*Notemigonus crysoleucas*), fathead minnow (*Pimephales promelas*), finescale dace (*Phoxinus neogaeus*), northern redbelly dace (*Phoxinus eos*), and brook stickleback (*Culaea inconstans*)). Mark–recapture population estimates of bass were conducted each year in May and August. Collectively, these lakes represent a broad range of prey to predator ratios, with Paul Lake at the low end of this gradient and Peter Lake at the high end. Bass in Peter Lake experienced prey abundances far exceeding those typical of lakes in this region. A summary of the population characteristics and samples from each lake is provided in Table 1. Although estimates of prey fish abundance are not available for most lakes, bass abundance is a good

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Table 1. Description of study lakes and largemouth bass population and individual sizes.

Lake	Lake size (ha)	No. years	No. consumption estimates	Mean (range) bass density (no.·ha^{-1})[b]	Mean (range) bass size (g)
Paul	1.5	13	174	142 (58–217)	198 (37–621)
Tuesday	1.2	2	107	375 (300–450)	211 (86–550)
West Long	3.4[a]	6	129	34 (4–71)	262 (38–1199)
Peter	2.2	1	17	38	219 (88–603)

[a]A neoprene curtain that divided West Long Lake was removed in the fall of 1995. As a result, West Long Lake area was 5.8 ha during the 1996 growing season.
[b]Mean and range of estimated densities across years.

index of prey fish abundance in these lakes because abundant bass populations rapidly extirpate prey fish (Carpenter et al. 1987).

Bioenergetic estimates of predation rates

Bioenergetics models estimate consumption by solving the energetic balance such that energy inputs equal outputs (Winberg 1956):

$$(5) \qquad C = G + R + \mathrm{SDA} + F + U + \mathrm{REP}$$

where C is consumption rate, G is fish growth, R is a temperature- and size-dependent respiration function, SDA is metabolic cost of digestion, F is egestion, U is excretion, and REP is energetic allocation to reproduction. The Wisconsin bioenergetics model (Kitchell et al. 1977) calculates C as the proportion (p) of the maximum consumption rate:

$$(6) \qquad C = pC_{\max}r(t)$$

where $r(t)$ is a temperature dependence function and $C_{\max}$ is the allometrically scaled maximum consumption rate (grams per gram per day). Parameters for the size and temperature dependence of C are derived from laboratory studies (Niimi and Beamish 1974). The model calculates the proportion of $C_{\max}$ that must have been consumed to solve the energy balance given observed fish growth rate, diet composition, and temperature. The parameter p in the bioenergetics model is equivalent to the parameter p used in the functional response model.

Individual bass growth rates were determined by measuring the growth of individually marked fish over the course of the growing season. Throughout the study period, bass >150 mm (maximum length = 490 mm) were marked with individually numbered floy tags. Because of the intense sampling of bass in these lakes, nearly all individuals >150 mm in each lake were marked. The earliest samples were taken shortly after ice-out in mid- to late May and the latest samples were taken at the end of August in each year. Fish were captured either by electrofishing or by angling. Total length (millimetres) and mass (grams) were measured on all fish at each capture.

Diet composition was determined from biweekly sampling of 12–25 (mean = 24) largemouth bass from each lake. Stomach contents were collected within 1 h of capture by flushing stomachs with a hand-pumped, pressurized backpack sprayer. Stomach contents were then passed through a 0.29-mm Nitex mesh concentrator, stored in individual vials, and preserved in 95% ethanol. Stomach contents were identified, enumerated, and grouped into 19 prey categories previously described by Hodgson et al. (1993). For each sampling date, we calculated the proportional contribution (by mass) of each diet group. Mass of each diet item categories was determined from the literature and from weighing subsamples of diet items. Energy density conversions for each diet group are presented in Hodgson et al. (1993). Water temperatures were measured weekly in each lake. We used the mean weekly epilimnetic temperature as input into the bioenergetics model.

We used the bioenergetics equations developed by Rice et al. (1983), and later validated by Rice and Cochran (1984), to estimate p for all marked individuals that were captured in both the May and August samples. For the 1996 Peter Lake bass, initial mass of each fish was known when stocked in early summer and we estimated p for all individuals captured during the August sample. In total, we estimated p for 427 individual bass, which included a broad range of bass sizes (Table 1). We assumed that the average diet composition at each sample date reflected the diet of the entire population. We also assumed that all bass >200 g reproduced on June 15 and allocated 10% of body energy to reproduction. This was a liberal estimate of energy allocation to reproduction but was chosen to encompass the largest values observed in largemouth bass (e.g., Miranda and Muncy 1987). Consequently, our estimates represent the largest possible predation rates.

We used a bootstrap procedure to generate frequency distributions of p for each lake. This procedure assured that our results were not biased by interannual variation in sampling intensity. Bootstrap samples were drawn (with replacement) using a two-staged sampling algorithm that biased the sampling towards observations taken in years in which bass were more abundant. The algorithm first selected a year from which the sample would be drawn such that the probability of selecting year i was equal to N_i/N_t, where N_i is the abundance during year i and N_t is the sum of the population abundances from that lake over the study period. The algorithm then randomly sampled one observation within that year. This was repeated 10 000 times for each lake, and the resulting data were used to generate frequency distributions of p.

Evaluating variability in daily predation rates

We estimated stomach content mass of all bass sampled during the study period (n = 3316) from diet counts and previously published mass conversions (Hodgson et al. 1993). We estimated the mass of young-of-year bass in diet samples from mean hatching dates and empirical estimates of young-of-year growth rates. We standardized stomach fullness (s) in the same way that we standardized consumption rate, i.e., we divided observed s by the s associated with $C_{\max}$ (Niimi and Beamish 1974). A detailed description of this calculation is presented in the Appendix. The same bootstrap procedure used for generating frequency distributions of p was used to generate frequency distributions of s.

To characterize the pattern of variability in daily consumption, we fit parameters of normal (nonpatchy) and gamma (patchy) distributions to data from each lake:

$$(7a) \quad \text{Normal:} \qquad f(s) = \frac{1}{\sqrt{2\pi\sigma^2}} \exp\left(-\frac{(s-\mu)^2}{2\sigma^2}\right)$$

$$(7b) \quad \text{Gamma:} \qquad f(s) = \frac{a^n}{\Gamma(n)} s^{n-1} \exp(-as).$$

The normal distribution is symmetric whereas the gamma distribution has a thick right tail and thereby replace the Poisson and the

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negative binomial distributions, respectively, yet allow s to be a continuous variable. When fitting gamma distributions, we added 0.01 to each estimate of s because gamma probability functions do not allow $s = 0$. We calculated maximum likelihood parameter estimates for both models and compared the likelihoods of the normal and gamma models using the likelihood ratio (Hilborn and Mangel 1997). We then estimated the coefficient of variation (CV) of the best-fitting model from the parameter estimates (normal = σ/μ, gamma = $n^{-0.5}$).

Results

Bioenergetic estimates of predation rates

We estimated predation rates for more than 100 largemouth bass in each of Paul, Tuesday, and West Long lakes and for 17 bass in Peter Lake (Table 1). Overall, predation rates of bass were low, with a mode of $p = 0.30$ and a mean of $p = 0.41$ (Fig. 2). Predation rates of Paul Lake bass were consistently low, with p ranging from 0.15 to 0.65. Further, 80% of the frequency distribution of p fell between 0.3 and 0.5. Tuesday Lake bass had similar predation rates; the mode of p was 0.3 and ranged from 0.20 to 0.65. Bass densities in these two lakes were the highest of all the study lakes (Table 1). West Long Lake bass exhibited a broad, bimodal range of predation rates with a large mode at $p = 0.4$ and a smaller mode at $p = 0.75$. Both of these values exceeded the modal values found in Paul and Tuesday lakes. The small, high mode was associated with a cohort of age 1+ bass in 1996 that grew rapidly from an average weight of 51 g in May to 225 g in August. Bass in Peter Lake had the highest modal consumption rate, $p = 0.5$. However, even in the presence of an abundant minnow population, predation rates in Peter Lake never exceeded $p = 0.6$.

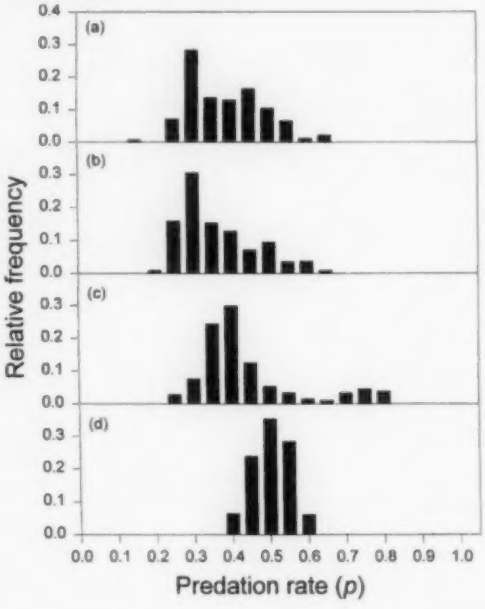
We identified sources of variation in p by calculating the average p for each lake and year and regressing this against bass density and mean bass mass. The latter variable was included because yearly variation in population size structure and concomitant changes in C_{max}, may have masked any apparent variation attributable to bass density. The regression model explained 34% of the variation in p, and the coefficients for both predictors were significant (density: slope = -4.18×10^{-3}, $t = 2.63$, $P = 0.017$; mass: slope = -4.54×10^{-3}, $t = 2.47$, $P = 0.023$). Thus, predation rates were significantly and negatively density dependent after correcting for differences in bass size. Because variation in bass biomass density was largely driven by variation in numerical abundance, we did not evaluate whether bass biomass added additional predictive power to the regression.

Variability in daily consumption

Instantaneous s was variable in Paul ($n = 2113$), Tuesday ($n = 364$), and West Long lakes ($n = 789$) (Fig. 3). In all of these lakes, the modal s was between 0 and 0.1. Further, s exceeded the maximum sustainable consumption rate (i.e., $s > 1$) for 10, 15, and 18% of bass in Paul, Tuesday, and West Long lakes, respectively. In contrast, the frequency distribution of s in Peter Lake ($n = 50$) was less patchy and had a considerably higher mode ($p = 0.35$). No bass from Peter Lake had $s > 1$.

There was little difference in the frequency distributions of s generated by bootstrap and the frequency distribution of s from the raw data, indicating that the variation in sampling

Fig. 2. Corrected frequency distributions of integrated predation rate estimated from the bioenergetics model in the four study lakes (*a*) Paul Lake, (*b*) Tuesday Lake, (*c*) West Long Lake, and (*d*) Peter Lake. Raw frequency distributions were adjusted to account for variation in sampling effort and fish abundance between years (see text for details).



between lakes and years closely paralleled the variation in bass abundance. We therefore fit normal and gamma parameters to the raw data. For all lakes, the data were best described by gamma (patchy) distributions (Table 2). For Paul, Tuesday, and West Long lakes, the likelihood of the gamma model was over 200 orders of magnitude greater than the likelihood of the normal model. For Peter Lake, the likelihood ratio was only 11.36, indicating that the probability of the observed data was 11 times greater given the gamma model than given the normal model. Thus, for all lakes, daily consumption was best described by a patchy probability distribution. The estimated CV for Paul (CV = 1.47), Tuesday (CV = 1.4), and West Long Lake (CV = 1.38) bass exceeded that estimated from Peter Lake bass (CV = 0.93) (Table 2).

Although s cannot be interpreted directly as the daily consumption rate (Elliot and Persson 1978), it can be used to evaluate variation in daily consumption provided that the gastric evacuation rates are not too high. When the evacuation rates are high, the instantaneous s is a poor indicator of the variability in daily prey captures because there is a greater probability that a meal consumed by a bass may not be present in the stomach when sampled later that day. We therefore examined the range of gastric evacuation rates calculated from the temperatures experienced by bass in this study. Epilimnetic temperatures ranged from 11 to 24.5°C corresponding to evacuation rates of 0.03–0.13·h^{-1}, expressed as the fraction of stomach contents evacuated each hour (Cochran and Adelman 1982). Thus, 50% of the mass of a meal would be present in the bass stomachs 23 and

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Fig. 3. Corrected frequency distributions of instantaneous stomach fullness in the four study lakes (*a*) Paul Lake, (*b*) Tuesday Lake, (*c*) West Long Lake, and (*d*) Peter Lake. Stomach fullness was scaled such that a value of 1 indicates the stomach fullness associated with the maximum sustainable consumption rate. Raw frequency distributions were adjusted to account for variation in sampling effort and fish abundance between years. Note the break in scale at $s = 1$.

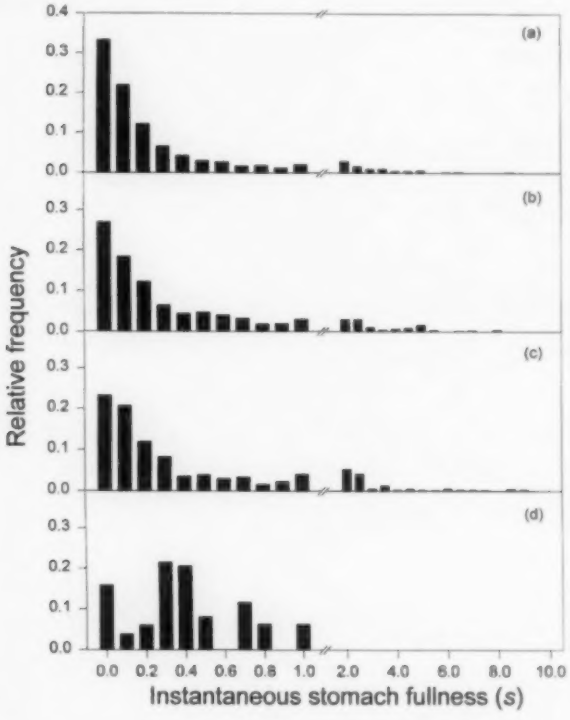


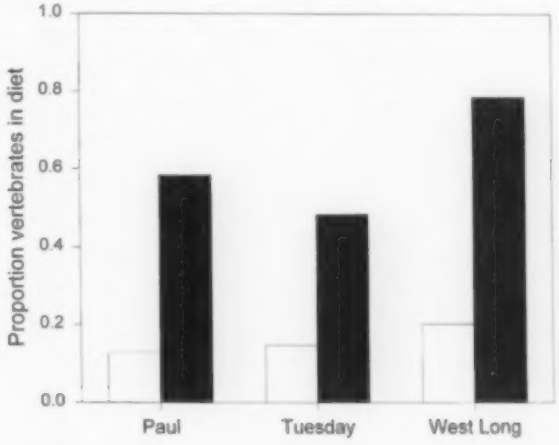
Table 2. Parameter estimates and negative log likelihood (–log(*L*)) for the normal and gamma model fits to stomach fullness data.

	Normal			Gamma		
Lake	μ	σ	–log(*L*)	N	*a*	–log(*L*)
Paul	0.56	4.0	5921.3	0.47	0.82	337.6
Tuesday	0.60	1.20	580.9	0.51	0.83	112.6
West Long	0.67	1.38	1372.5	0.52	0.77	334.4
Peter	0.39	0.28	6.9	1.16	2.88	4.4

5.5 h after consumption at the coolest and warmest temperatures, respectively, and 25% would be present 46 and 10 h after consumption. Thus, it is unlikely that the large number of empty stomachs ($s = 0$–0.1) was the result of rapid digestion.

We then evaluated which prey items were being consumed when $s > 1$. Specifically, we determined the proportion (by mass) of vertebrates in the stomach samples (terrestrial vertebrates, young-of-year bass, yearling bass, other fish) of bass that had $s < 1$ and $s \geq 1$. We did not consider Peter Lake in this analysis because there were no samples for which $s \geq 1$. The average proportion of vertebrates in the diets when $s > 1$ was significantly greater than when $s < 1$

Fig. 4. Comparison of diet composition between largemout bass whose stomach content mass exceeded that predicted from C_{max} ($s > 1$, solid bars) and bass whose stomach content mass was less than predicted from C_{max} ($s < 1$, open bars). Proportion of vertebrates refers to the proportion (by mass) of terrestrial vertebrates, young-of-year bass, yearling bass, and other fish in the diets.



(ANOVA of log-transformed *s*: $F_{1,2878} = 166$, $P < 0.001$) (Fig. 4). Thus, bass were more likely to have instantaneous predation rates that exceeded the maximum sustainable rates when consuming large prey items such as fish and terrestrial vertebrates.

Discussion

Predation rates of largemouth bass were generally low and daily prey consumption was variable. If we interpret these results in light of our functional response model, the initial conclusion would be that satiation was a moderate constraint on the mean predation rate ($p = 0.3$) because at that point on the functional response curve, predation rates are 20% less than the potential predation rate (assuming CV = 1.4) (Fig. 1). However, this model prediction was based on the assumption that the daily C_{max} is constant and equal to the maximum sustainable consumption rate, i.e., instantaneous satiation = integrated satiation. Our data indicate that this assumption is not valid, as nearly 13% of all bass had more food in their stomachs than predicted from the maximum sustainable consumption rates (Niimi and Beamish 1974).

Our revised view of the functional response model is that at low mean prey densities, daily predation rates can greatly exceed the maximum sustainable predation rates (instantaneous satiation >> integrated satiation). Consequently, variability in prey encounters did not lead to reduced predation rates, i.e., opportunistic feeding shifted the functional response curve upwards. If the functional response curve is shifted up such that the curve denoted by random prey encounters is the appropriate model, then the modal consumption rate ($p = 0.3$) is on a portion of the functional response that does not deviate from the potential predation rate (Fig. 1). Further, only 18 of the 427 individual consumption rates were large enough ($p > 0.66$) to expect that a 10%

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reduction in predation rates occurred due to satiation. Thus, we conclude that bass predation rates were rarely constrained by time costs of digesting prey. Of course, we do not know the extent to which the opportunistic consumption capabilities of bass can mitigate short-term satiation or the range of mean prey encounters for which opportunistic predation may be feasible. However, given that some estimates of s were several times that predicted from the sustained C_{max}, we suspect that opportunistic consumption can play a large role in reducing the constraint of instantaneous satiation when prey encounters are rare and highly patchy.

There are at least three explanations for the low predation rates reported here. The first is that our estimates of p were incorrect. One potential source of error is that the laboratory-derived estimates of C_{max} (Niimi and Beamish 1974) are not correct or they are not applicable in natural settings. We know of no evidence to support these prospects. The data collected by Niimi and Beamish (1974) are among the most thorough measurements of C_{max} for any fish, so it is unlikely that there are large errors in these estimates. Also, largemouth bass are top predators in these lakes and are therefore unlikely to face the same foraging/predation risk trade-off that smaller fish must face (Lima and Dill 1990). Another possibility is that the bioenergetics model grossly underestimated consumption rates. This too is unlikely, as Rice and Cochran (1984) showed strong correspondence between bass predation estimates derived from the bioenergetics model and those derived from an independent field study. Specifically, bioenergetics-based estimates had only an 8% error when using the same sampling frequency for diet analyses and water temperature as input into the bioenergetics calculation. Moreover, Rice and Cochran's (1984) evaluation was based on data collected in the same lakes as our studies and during similar seasons. Additionally, we used a liberal estimate of energy allocation to reproduction, so if anything, our predation estimates are biased upwards.

A second explanation is that bass predation drastically reduces prey abundance, and as a consequence, prey encounter rates are rarely large enough for satiation to become important. This explains the fact that predation rates were consistently lowest in the two lakes containing the highest bass densities, Paul and Tuesday lakes. Highly profitable prey were rare in these lakes and bass diets were dominated by benthic invertebrates (Schindler et al. 1997). Moreover, the significant negative correlation between bass density and p is consistent with the prey depletion hypothesis. However, prey depletion does not explain the low predation rates in Peter Lake. Eighty-four bass were introduced into Peter Lake in mid-July, which at that time contained an abundant minnow population (>40 000 g·ha^{-1} based on night purse-seine catches). This prey abundance greatly exceeds that normally experienced by bass in north temperate lakes, yet consumption rates averaged only $p = 0.5$ in Peter Lake. Similarly, brook stickleback were highly abundant in West Long Lake in 1995 and 1996, yet with the exception of small bass (<50 g), p did not exceed 0.6. Thus, although predator depletion of prey can explain some of the patterns observed here, other processes must also be operating to keep predation rates low.

The third possibility is that some other constraint operates that effectively reduces predation rates below those expected by predator satiation. One such constraint may be predator and prey behavior. Prey fish in these lakes exhibit strong behavioral responses to bass (He and Kitchell 1990), which reduce their vulnerability to predation (He et al. 1993). Essington (1999) showed that the spatial distributions of bass and their prey are strongly and negatively correlated across a broad range of spatial scales. Essington (1999) inferred from this that the behavioral response of prey to bass is more important than the prey-seeking response of predators when predation rates are high. These behavioral responses can play an important role in governing predation rates, as Walters et al. (1997) showed that an asymptotic functional response curve can be derived from simple assumptions regarding prey refuges and other antipredator behavior. We assert that antipredator behavior is an important constraint on predation rates in natural systems because it effectively reduces the maximum rate per predator to a level much lower than that that might be derived from experimental studies conducted in a confined space.

The ability of piscivores to extirpate small-bodied fishes in north temperate lakes has been well documented (Tonn and Magnuson 1982). Our analyses demonstrate that one possible reason for this phenomenon is that when prey fish are available, there is little physiological constraint on the rate at which piscivores consume prey. Consequently, prey must rely on predator-avoidance behaviors, yet these behaviors may make prey vulnerable to other mortality agents such as avian predators or starvation (He and Kitchell 1990). Schindler et al. (1997) proposed that the ability of bass to maintain numerical dominance in the absence of small-bodied fishes is a consequence of the opportunistic feeding strategy of bass, which enables bass to maintain high densities in the absence of preferred prey. Our data indicate that bass are opportunistic in another manner in that they can consume prey at high rates for short periods of time. This has two important implications. The first is that bass are able to sustain higher predation rates than they would otherwise, i.e., the functional response is shifted upwards. The second is that when small-bodied fish become available, bass can consume them at rates far exceeding their maximum sustainable consumption rates. Evidence supporting this comes from our analyses of diet compositions during instances of opportunistic foraging ($s > 1$): bass diets contained significantly higher proportions of fish and other vertebrates when $s > 1$. Thus, it is highly unlikely that small-bodied fishes can recolonize these lakes following extirpation because predation rates during colonization events are probably very intense.

One potential analytical error is that we overestimated s by underestimating S_{max}, the stomach fullness associated with maximum sustained ration (C_{max}). We believe that our results represent a real phenomenon. Daily consumption rates of bass are inherently variable, even when presented with constant prey availability (Smagula and Adelman 1982). Also, the differences in the distribution of s between the lakes are consistent with the hypothesis that opportunistic foraging is a behavioral adaptation to rare prey encounters. Specifically, bass in Paul, Tuesday, and West Long lakes, all of which had relatively low prey abundances, frequently had values of $s > 1.0$ whereas this never occurred for bass in Peter Lake. Daily predation rates in Peter Lake were fairly constant over the study period, suggesting that these

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bass did not have to rely on brief periods of high predation rates. Further, the fact that s never exceeded 1 in Peter Lake makes it unlikely that the high estimates of s in the other lakes were due to a systematic analytic error. Thus, we believe that this "binge-feeding" phenomenon reflects a behavioral and biophysical adaptation to environments in which encounters with highly profitable prey are uncommon.

There is considerable evidence that satiation may be an uncommon phenomenon for piscivorous fishes. Lewis et al. (1974) found that nearly one half of 991 largemouth bass sampled over an 18-month period in an Illinois reservoir had empty stomachs at the time of sampling, and DeAngelis et al. (1984) found that largemouth bass rarely had more than one prey item in their stomachs. Madenjian et al. (1998) reported that predation rates of lake trout (*Salvelinus namaycush*) in two reefs in Lake Michigan were less than $p = 0.3$. Finally, a recent survey of predation rates of 68 piscivorous fish species (Schindler and Eby 1997) indicated that fish growth rates were <40% of their physiological maximum rate in 60% of the cases (D.E. Schindler, University of Washington, Seattle, Wash., personal communication). In general, it appears that foraging rates of piscivores may not be limited by physiological constraints. In other words, the satiation effects widely represented in modeling studies may be a rare component in piscivore–prey interactions whereas predator and prey behavior may play a more important role.

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References

Breck, J.E. 1993. Foraging theory and piscivorous fish: are forage fish just big zooplankton? Trans. Am. Fish. Soc. **122**: 902–911.

Carpenter, S.R., and Kitchell, J.F. 1987. The temporal scale of variance in limnetic primary production. Am. Nat. **129**: 417–433.

Carpenter, S.R., Kitchell, J.F., and Hodgson, J.R. 1985. Cascading trophic interactions and lake productivity. BioScience, **35**: 634–639.

Carpenter, S.R., Kitchell, J.F., Hodgson, J.R., Cochran, P.A., Elser, J.J., Elser, M.M., Lodge, D.M., Kretchmer, D., He, X., and von Ende, C.N. 1987. Regulation of lake primary productivity by food web structure. Ecology, **68**: 1863–1876.

Cochran, P.A., and Adelman, I.R. 1982. Seasonal aspects of daily ration and diet of largemouth bass, *Micropterus salmoides*, with an evaluation of gastric evacuation rates. Environ. Biol. Fishes, **7**: 265–275.

DeAngelis, D.J., Adams, S.M., Breck, J.E., and Gross, L.J. 1984. A stochastic predation model: application to largemouth bass (*Micropterus salmoides*) observations. Ecol. Model. **24**: 25–42.

Elliot, J.M., and Persson, L. 1978. The estimation of daily rates of food consumption for fish. J. Anim. Ecol. **47**: 977–991.

Essington, T.E. 1999. Spatial dynamics, stage-structure, and the predator–prey interactions of largemouth bass (*Micropterus salmoides*). Ph.D. dissertation, University of Wisconsin-Madison, Madison, Wis.

Hall, D.J., and Ehlinger, T.J. 1989. Perturbation, planktivory, and pelagic community structure: the consequence of winterkill in a small lake. Can. J. Fish. Aquat. Sci. **46**: 2203–2209.

Hassell, M.P. 1978. Dynamics of arthropod predator–prey systems. Princeton University Press, Princeton, N.J.

He, X., and Kitchell, J.F. 1990. Direct and indirect effects of predation on a fish community: a whole-lake experiment. Trans. Am. Fish. Soc. **119**: 825–835.

He, X., Kitchell, J.F., Hodgson, J.R., Wright, R.A., Soranno, P.A., Lodge, D.M., Cochran, P.A., Benkowski, D.A., and Bouwes, N.W. 1993. Roles of fish predation: piscivory and planktivory. *In* The trophic cascade in lakes. *Edited by* S.R. Carpenter and J.F. Kitchell. Cambridge University Press, Cambridge, U.K. pp. 85–102.

Hilborn, R., and Mangel, M. 1997. The ecological detective: confronting models with data. Princeton University Press, Princeton, N.J.

Hodgson, J.R., and Kitchell, J.F. 1987. Opportunistic feeding of largemouth bass (*Micropterus salmoides*). Am. Midl. Nat. **118**: 323–335.

Hodgson, J.R., He, X., and Kitchell, J.F. 1993. The fish populations. *In* The trophic cascade in lakes. *Edited by* S.R. Carpenter and J.F. Kitchell. Cambridge University Press, Cambridge, U.K. pp. 43–48.

Holling, C.S. 1959. Some characteristics of simple types of predation and parasitism. Can. Entomol. **91**: 385–398.

Kitchell, J.F., Stewart, D.J., and Weininger, D. 1977. Application of a bioenergetics model to yellow perch (*Perca flavescens*) and walleye (*Stizostedion vitreum vitreum*). J. Fish. Res. Board Can. **34**: 1922–1935.

Lewis, W.M, Heidinger, R., Kirk, W., Chapman, W., and Johnson, D. 1974. Food intake of the largemouth bass. Trans. Am. Fish. Soc. **103**: 277–280.

Lima, S.L., and Dill, L.M. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. Can. J. Zool. **68**: 619–640.

Mackenzie, B.R., Leggett, W.C., and Peters, R.H. 1990. Estimating larval fish ingestion rates: can laboratory derived values be reliably extrapolated to the wild? Mar. Ecol. Prog. Ser. **67**: 209–225.

Madenjian, C.P., DeSorcie, T.J., and Stedman, R.M. 1998. Ontogenic and spatial patterns in diet and growth of lake trout in Lake Michigan. Trans. Am. Fish. Soc. **127**: 236–252.

May, R.M. 1977. Thresholds and breakpoints in ecosystems with a multiplicity of stable states. Nature (Lond.), **269**: 471–477.

Miranda, L.E., and Muncy, R.J. 1987. Recruitment of young-of-year largemouth bass in relation to size structure of parental stock. N. Am. J. Fish. Manage. **7**: 131–137.

Mittelbach, G.G., Turner, A.M., Hall, D.J., Rettig, J.E., and Osenberg, C.W. 1995. Perturbation and resilience: a long-term whole-lake study of predator extinction and reintroduction. Ecology, **76**: 2347–2360.

Murdoch, W.W. 1994. Population regulation in theory and practice. Ecology, **75**: 271–287.

Murdoch, W.W., and Oaten, A. 1975. Predation and population stability. Adv. Ecol. Res. **9**: 1–131.

Niimi, A.J., and Beamish, F.W.H. 1974. Bioenergetics and growth of largemouth bass (*Micropterus salmoides*) in relation to body weight and temperature. Can. J. Zool. **52**: 447–456.

Ramcharan, C.W., McQueen, D.J., Demers, E., Popiel, S.A., Rocchi, A.M., Yan, N.D., Wong, A.H., and Hughes, K.D. 1995. A comparative approach to determining the role of fish predation in structuring limnetic ecosystems. Arch. Hydrobiol. **133**: 389–416.

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Rice, J.A., and Cochran, P.A. 1984. Independent evaluation of a bioenergetics model for largemouth bass. Ecology, **65**: 732–739.

Rice, J.A., Breck, J.E., Bartell, S.M., and Kitchell, J.F. 1983. Evaluating the constraints of temperature, activity and consumption on growth of largemouth bass. Environ. Biol. Fishes, **9**: 263–275.

Schindler, D.E., and Eby, L.A. 1997. Interactions between growth rates and stoichiometry of fishes and their prey: implications for nutrient recycling. Ecology, **78**: 1816–1831.

Schindler, D.E., Hodgson, J.R., and Kitchell, J.F. 1997. Density-dependent changes in individual foraging specialization of largemouth bass. Oecologia, **110**: 592–600.

Smagula, C.M., and Adelman, I.R. 1982. Day-to-day variation in food consumption by largemouth bass. Trans. Am. Fish. Soc. **111**: 543–548.

Solomon, M.E. 1949. The natural control of animal populations. J. Anim. Ecol. **18**: 1–35.

Tonn, W.M., and Magnuson, J.J. 1982. Patterns in the species composition and richness of fish assemblages in northern Wisconsin lakes. Ecology, **63**: 1149–1166.

Walters, C., Christensen, V., and Pauly, D. 1997. Structuring dynamic models of exploited ecosystems from trophic mass-balance assessments. Rev. Fish Biol. Fish. **7**: 39–172.

Winberg, G.G. 1956. Rate of metabolism and food requirements of fishes. Belorussian Univ. Minsk. (Transl. by Fisheries Research Board of Canada, Transl. Ser. No. 164, 1960.)

Appendix

We sought to determine the mass of food in bass stomachs at the time that feeding ceases due to satiation (S_{max}) under the assumption that instaneous satiation equals integrated satiation. To this end, we used the maximum sustainable consumption rates measured by Niimi and Beamish (1974) and the gastric evacuation model presented by Cochran and Adelman (1982). The gut evacuation rate is described by an exponential model (Beamish 1972):

$$\text{(A1)} \quad S_t = S_0 \exp(-\alpha t)$$

where S_t is the mass of stomach contents at time t, S_0 is the initial stomach content mass, and α is a temperature-dependent evacuation rate. Cochran and Adelman (1982) fitted an exponential model for the temperature dependence of α, and we used this empirically derived relationship when calculating S_{max} for each bass:

$$\text{(A2)} \quad \alpha = \exp(1.7 \ln(T) - 7.455).$$

The S_{max} can be determined from the maximum consumption rate (C_{max}), and the manner by which bass were fed to determine C_{max}. The C_{max} estimates in our model were based on the experiments of Niimi and Beamish (1974) wherein bass were presented with an abundance of minnows and were considered satiated when no feeding was observed for 10 min. This procedure was conducted twice daily at approximately 12-h intervals to generate C_{max}.

If we assume that at each feeding, bass consume $0.5C_{max}$ and that evacuation during each feeding bout is negligible, then the stomach mass associated with cessation of feeding is

$$\text{(A3)} \quad S_{max} = 0.5C_{max}(e^{-12\alpha} + 1).$$

In other words, the amount of food in the stomach of a bass in the experiment of Niimi and Beamish (1974) at the cessation of feeding equaled the amount of food just consumed ($0.5C_{max}$) plus the food remaining from the feeding bout 12 h earlier ($0.5C_{max}e^{-12\alpha}$).

We calculated S_{max} for each bass sampled. Temperature estimates were taken from concurrent limnological sampling, and we used the mean epilimnetic temperature sample taken on the day closest to the day that each bass was sampled. The number of days between temperature and bass samples never exceeded 4.

For each diet sample, we calculated the standardized stomach fullness (s), which is defined as

$$\text{(A4)} \quad s = S_{obs}/S_{max}$$

where S_{obs} is the observed stomach content mass. Thus, s is scaled in a manner so that it is comparable with p, the standardized integrated predation rate.

Appendix references

Beamish, F.W.H. 1972. Ration size and digestion in largemouth bass, *Micropterus salmoides* Lacépède. Can. J. Zool. **50**: 153–164.

Cochran, P.A., and Adelman, I.R. 1982. Seasonal aspects of daily ration and diet of largemouth bass, *Micropterus salmoides*, with an evaluation of gastric evacuation rates. Environ. Biol. Fishes, **7**: 265–275.

Niimi, A.J., and Beamish, F.W.H. 1974. Bioenergetics and growth of largemouth bass (*Micropterus salmoides*) in relation to body weight and temperature. Can. J. Zool. **52**: 447–456.

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Direct behavioral evidence that unique bile acids released by larval sea lamprey (*Petromyzon marinus*) function as a migratory pheromone

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Abstract: Four behavioral experiments conducted in both the laboratory and the field provide evidence that adult sea lamprey (*Petromyzon marinus*) select spawning rivers based on the odor of larvae that they contain and that bile acids released by the larvae are part of this pheromonal odor. First, when tested in a recirculating maze, migratory adult lamprey spent more time in water scented with larvae. However, when fully mature, adults lost their responsiveness to larvae and preferred instead the odor of mature individuals. Second, when tested in a flowing stream, migratory adults swam upstream more actively when the water was scented with larvae. Third, when migratory adults were tested in a laboratory maze containing still water, they exhibited enhanced swimming activity in the presence of a 0.1 nM concentration of the two unique bile acids released by larvae and detected by adult lamprey. Fourth, when adults were exposed to this bile acid mixture within flowing waters, they actively swam into it. Taken together, these data suggest that adult lamprey use a bile acid based larval pheromone to help them locate spawning rivers and that responsiveness to this cue is influenced by current flow, maturity, and time of day. Although the precise identity and function of the larval pheromone remain to be fully elucidated, we believe that this cue will ultimately prove useful as an attractant in sea lamprey control.

Résumé : Quatre expériences sur le comportement, réalisées en laboratoire et sur le terrain, ont montré que la grande lamproie marine (*Petromyzon marinus*) adulte choisit sa frayère selon l'odeur des larves qui s'y trouvent, et que les acides biliaires excrétés par les larves jouent un rôle dans la production de cette odeur phéromonale. Premièrement, lors de tests dans un labyrinthe d'eau recirculée, les lamproies adultes migratrices ont passé plus de temps dans l'eau imprégnée de l'odeur des larves. Cependant, à pleine maturité, les adultes ont perdu leur sensibilité à l'odeur des larves et ont préféré l'odeur des autres lamproies adultes. Deuxièmement, lors de tests dans un cours d'eau, les adultes migrateurs nageaient vers l'amont plus vigoureusement lorsque l'eau était imprégnée de l'odeur des larves. Troisièmement, lorsque les adultes migrateurs ont été placés en laboratoire dans un labyrinthe d'eau calme, ils nageaient plus vite en présence d'une concentration de 0,1 nM des deux acides biliaires pariculiers excrétés par les larves et détectés par les lamproies adultes. Quatrièmement, lorsque les adultes étaient exposés à ce mélange d'acides biliaires en eau courante, ils nageaient plus vigoureusement vers la source d'exposition. En rassemblant ces données, nous pouvons penser que la lamproie adulte utilise une phéromone larvaire à base d'acide biliaire pour situer les frayères, et que la sensibilité à ce signal est influencée par le débit d'eau, la maturité et le moment de la journée. Même si l'identité et la fonction de la phéromone larvaire restent à déterminer avec précision, nous pensons que cet indice peut être très utile en tant qu'attractif pour lutter contre la grande lamproie marine.

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Introduction

The sea lamprey (*Petromyzon marinus*) is an anadromous, jawless fish with a complex and dramatic life history. Native to the Atlantic Ocean, this species entered the Great Lakes at the turn of the century; there, it functions as a destructive parasite that threatens many fish populations (Smith and Tibbles 1980). In the spring, adult sea lamprey migrate from the ocean (or lakes) into tributary streams where they construct nests for spawning. The young hatch as blind larvae that live in the stream sediments, filter-feeding on detritus. Upon reaching a critical size of about 150 mm, a process that may take from 3 to 18 years (Purvis 1980), larvae metamorphose into a parasitic phase, migrate downstream into either the lake or the ocean, and feed on other fish. In the Great Lakes, parasitic lamprey feed for approximately 15 months, increasing 100-fold in body mass, at which time they stop feeding, start to mature sexually, and enter a migratory phase during which they search for spawning rivers (Hardisty and Potter 1971). Upstream spawning migration is dramatic and brief (4–8 weeks), associated with continuing sexual maturity and culminating in spawning and death (Applegate 1950).

Stream selection by migratory adult sea lamprey in the Great Lakes appears specific, as less than 10% of the 6000 tributaries entering the Great Lakes presently support larval populations (Morman et al. 1980; Annual Reports of the Great Lakes Fisheries Commission, 2100 Commonwealth Blvd., Ann Arbor, Mich.). However, the majority of the tributaries lacking sea lamprey are accessible to migratory adults and appear capable of supporting larval populations; indeed, many did prior to lamprey control when adult numbers were much higher and larval abundance was unregulated (Torblaa and Westman 1980). Furthermore, tagging studies have clearly demonstrated that stream selection cannot be attributed to animals returning to their natal streams (Bergstedt and Seelye 1995). It seems likely that selection of spawning streams is based on innate preferences for particular environmental cues.

The hypothesis that adult lamprey select streams based on the presence of conspecific odor (pheromones) arose from an intensive analysis of the capture records of eight Lake Superior streams over a 20-year period (Moore and Schleen 1980). This study found that when larvae were removed from populated streams by treatment with the lampricide 3-trifluoromethyl-4-nitrophenol (TFM), the number of adults entering these streams dropped by about half the following year. This drop could not be attributed to elimination of that year-class, as all streams used in the analysis had been treated after that year's year-class had migrated to the lake. Subsequent support for pheromone-based migration has come from a preliminary behavioral experiment, which found that adult lamprey are not repelled by TFM and that larval odor can attract adults in the laboratory (Teeter 1980). A complete description of the latter results has not been published and is included in this article.

More recently, our physiological studies of the lamprey olfactory system have provided compelling evidence that bile acids might be a key component of a lamprey migratory pheromone. Briefly, we have discovered that larval sea lamprey produce and release two unique bile acids (petromyzonol sulfate (PS) and allocholic acid (ACA)) and that both of these compounds are detected with exquisite sensitivity (picomolar detection thresholds) and specificity by the olfactory system of migratory adults (Li et al. 1995; Li and Sorensen 1997). PS is of special interest because it has not been described in any vertebrate other than lamprey (Hoshita 1985; Polkinghorne 1997). Our ongoing laboratory analysis has succeeded in identifying PS at concentrations of about 10^{-10} M in tributaries of the Great Lakes with resident populations of larval sea lamprey (P.W. Sorensen, unpublished results).

We have been actively pursuing the hypothesis that PS and ACA together function as a migratory pheromone in the sea lamprey, and this paper presents the first behavioral test of this possibility. Although we are not the first to ask whether bile acids might function as pheromones in migratory fishes (Døving et al. 1980; Selset and Døving 1980), we are the first to test the behavioral activity of the specific bile acid compounds known to be released and detected by the species in question. It is important to clarify how we employ the term "pheromone" in this article, for this term has been used in many ways in the past. Here, we define a pheromone as an organismally derived odor that stimulates specific, innate, and adaptive responses in exposed conspecifics. This definition is based on that originally proposed by Karlson and Lüscher (1959) for insects and expanded by us with our discovery that fish use mixtures of relatively common hormonal metabolites as sex pheromones (Stacey and Sorensen 1991; Sorensen et al. 1998).

The present study asked two questions. (*i*) Does the odor of larval sea lamprey influence the distribution and swimming activity of migratory sea lamprey in a manner consistent with its proposed function as a migratory pheromone? (*ii*) Does the odor of larval bile acids (PS and ACA) influence the distribution and activity of migrating sea lamprey in a manner similar to that seen for larval odor when tested at relevant concentrations? Four experiments were conducted, each addressing these question(s) in slightly different manners using different designs. The studies started 15 years ago, prior to our formulation of the bile acid hypothesis. Experiment 1 examined larval odors while also examining the effect of gender, sexual maturity, and time of day on responsiveness. Experiment 2 tested larval odor in the field. The last two experiments focused on bile acids and were conducted in the laboratory because we lacked large quantities of these compounds. Experiment 3 tested lamprey bile acids in still water and Experiment 4 tested them in flowing water, thus allowing us to address the role of current in behavioral responsiveness. We now describe each experiment, giving the materials and methods and results for each. A general discussion summarizes all experiments and their significance.

Experiment 1. When offered the choice of slowly flowing water with and without larval sea lamprey, do adult sea lamprey select the former, and is this response life-stage specific?

The primary objective of this laboratory experiment was to confirm the results of a pilot study that suggested that the

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odor of larvae attracts migratory adults (Teeter 1980). A secondary objective was to ascertain whether sexually mature lamprey continue to respond to larval odor or whether they instead become responsive to the odor of adults (e.g., sex pheromones). A tertiary objective was to determine whether both males and females responded equally. All together, 10 experimental conditions were tested between 1980 and 1983 using the recirculating maze employed in our earlier pilot work (Teeter 1980; Lisowski et al. 1986). A preliminary account of one of these 10 experiments (the test of the responsiveness of immature males to larval odor) has been published (Teeter 1980).

Materials and methods

Migratory sea lamprey were trapped during April and May (1980–1983) from the inlet of Lake Cayuga (Ithaca, N.Y.) and the Ocqueoc and Cheboygan rivers (Michigan) and transported to either the Monell Chemical Senses Center (Philadelphia, Pa.) or the Hammond Bay Biological Station (Millersburg, Mich.) for testing. The majority of the lampreys captured during this period had not developed secondary sex characteristics (a dorsal ridge or "rope" in males and a thickening of the anal fin in females) and were considered to be "sexually immature" and still in the migratory phase. Once these secondary sex characteristics were apparent, animals were classified as "sexually mature." A few mature animals were spermiated or ovulated. Prior to testing, fish were maintained in same-sex groups of six in tanks supplied with 10–15°C well (Monell Chemical Senses Center) or Lake Huron water (Hammond Bay Biological Station). Immature sea lamprey were tested early in the season (May) and mature animals at the end (July).

Experiments were conducted in the recirculating two-choice maze (Fig. 1) used and described by Lisowski et al. (1986). This recirculating design was employed because the water supply was limited. Through the use of recirculating pumps and a barrier restriction, this device was able to maintain uniform odor concentrations down both channels while allowing animals to move between them at the far end where the main drains were located. Newly inflowing water was added to the upper end of each chamber at a rate of 200 mL·min^{-1}, and odor solution was added to one of these flows when or as appropriate. Current velocity in each arm was 10 cm·min^{-1} and a 40-W ultraviolet light was suspended over the common end of the tank to help observers see lamprey, which were individually marked with color-coded fluorescent tags inserted into their musculature (Lisowski et al. 1986).

To start an experiment, a group of six marked lamprey was placed into the lower end of the test apparatus and given 10 min to habituate. During this time, animals were allowed to swim freely throughout the tank. At end of this period, odor was added through one of the well/lake water inlets and an additional 5-min period was allowed to pass to permit the odor to mix throughout the experimental channel. In most cases, animals were seen to explore both channels during this stimulus-equilibration period; however, if this was not observed, the animal(s) was gently forced into the unexplored compartment using a net. At the end of the latter period, the experiment formally commenced and the positions of all six lamprey were continuously tracked and time spent

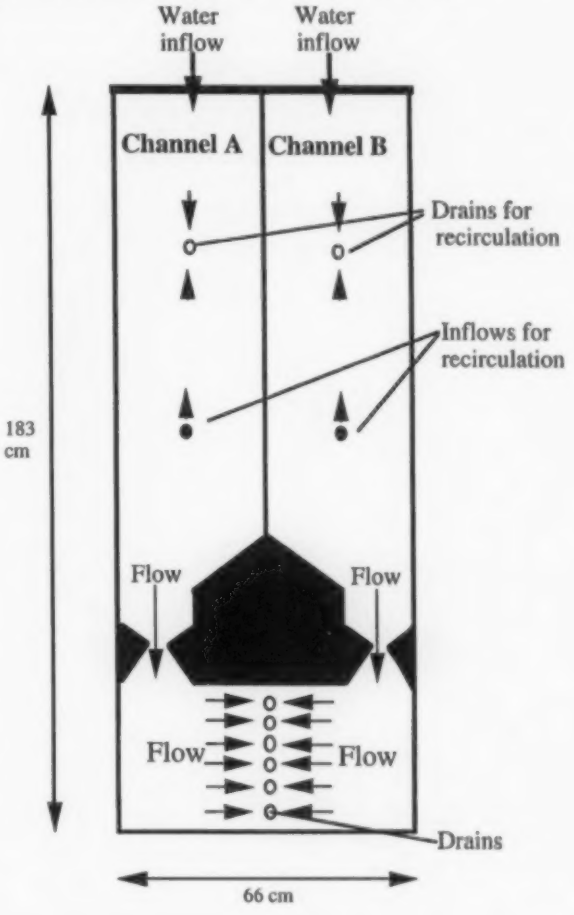

Fig. 1. Diagrammatic representation of the recirculating laboratory maze used to test sea lamprey in Experiment 1. Flow is indicated by arrows. Very slow current regimes were used. Six individually marked animals were tested at a time. A detailed plan of this device is given in Lisowski et al. (1986).

in each channel recorded using a bank of six stopwatches. To encourage swimming activity (lamprey tended to attach to the sides of the tank), a 100-W incandescent light, positioned over the center of the tank, was turned on for 30-s periods at 5-min intervals throughout the experiment. The temperature of the inflowing water and the water in both channels varied between 12 and 18°C (according to season), but they were always within 0.5°C of each other. Experiments lasted 30 min.

Responsiveness of mature and immature lamprey to the odor of larvae was tested first. Later, in a separate set of experiments, the effects of adult male and female odor were tested. Larval odor was created by holding 36 larvae in a 38-L tank. Control odor was created using an identical tank that contained sand only. In tests of female lamprey behavior, larval odor was tested after 144 h of holding; for males, a 24-h time period was used because of technical difficulties associated with holding larvae for so long. The odor of sexually immature and sexually mature male and female lamprey

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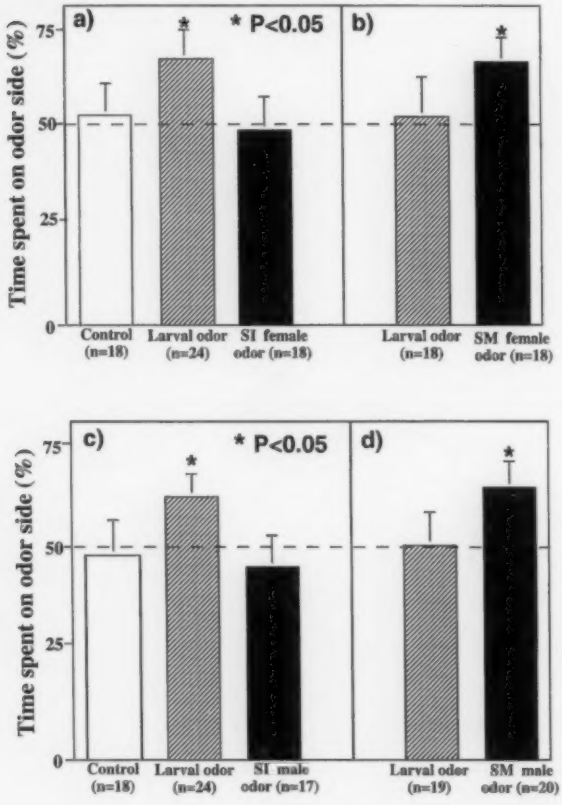
Fig. 2. Results of Experiment 1: mean percent time (±SE) spent by individual adult sea lamprey in various odors. Responses of four types of adult lamprey were tested: (*a*) sexually immature males, (*b*) sexually mature males, (*c*) sexually immature females, and (*d*) sexually mature females. Six odors were tested: control, odor of water from a tank containing sand only; larval odor, water from a tank containing larvae living in sand; SI (sexually immature) male odor, water from a tank containing sexually immature adult males; SI female odor, water from a tank containing sexually immature adult females; SM (sexually mature) male odor, water from a tank containing sexually mature males; SM female odor, water from a tank containing sexually mature females. Statistical comparisons are between times spent in the experimental odor and 50%, the time expected by chance. The number of individuals tested (*n*) is given in parentheses.



was created by holding single adults in 1 L of water for 1 h. The channel used for odor addition was selected at random (using a random number table) for each trial. In all trials, 4-L aliquots of these samples were added to the test channel. For analysis, the percent time spent by each lamprey in the test odor was compared with the expected value of 50% using the Wilcoxon matched-pairs, signed-ranks test (two-tailed; Instat, San Diego, Calif.). Each lamprey was treated as an independent sample because there was no indication of interactions within groups (Lisowski et al. 1986).

Results

Both sexually immature male (Fig. 2*a*) and sexually immature female lamprey (Fig. 2*c*) spent more time in larval holding water than they did in unscented water ($P < 0.05$). These animals also exhibited no response to either the odor of the sand control or the odor of sexually immature conspecifics of the opposite sex. In contrast, once sexually mature, neither males (Fig. 2*b*) nor females (Fig. 2*d*) responded to larval odor, but they did spend more time in the odor of sexually mature individuals of the opposite sex ($P < 0.05$).

Experiment 2. Does the odor of larval sea lamprey stimulate upstream swimming activity in migratory sea lamprey held in a naturally flowing stream?

This field experiment was conducted in flowing waters of streams known to be suitable for lamprey colonization. Its primary objective was to extend the results of Experiment 1 by testing whether migratory lamprey swim upstream with greater vigor when exposed to larval odor in flowing, natural waters. A secondary objective was to ascertain whether this response might be strongest at night when lamprey naturally swim upstream. A tertiary objective was to confirm that the behavioral response to larval odor weakens with the onset of sexual maturity.

Materials and methods

This experiment was conducted in May–June 1993 using a custom-made wooden maze anchored to a stream bottom. Experiments commenced in the Black Mallard River (Michigan), a small stream that normally supports a significant lamprey run but did not at the time because it had been treated with lampricide to remove larvae the year before. However, a flood occurred in late May and caused us to move to a nearby river (Grand Lake Outlet, Michigan) for the third test. Grand Lake Outlet was similar in size to the Black Mallard River and electrofishing surveys revealed it to have no larval lamprey at that time. Lamprey for all tests were captured in U.S. Fish and Wildlife Service traps located in the neighboring Cheboygan River and transported to the test site, where they were held in large plastic cages in shady areas of the river for 1–3 days prior to testing. Only females were used because males were needed for an experimental sterilization program, and Experiment 1 demonstrated that gender does not influence preference for larval odor. Animals used in the first two sets of experiments were captured and tested during the first week of their migration (May 15); they lacked secondary sexual characteristics and their gonadosomatic index (gonad weight divided by body weight × 100) was relatively small: 14.2 ± 0.4% (n = 12). Animals used in the third set of experiments were trapped and tested during the final week that significant numbers of animals were caught in Cheboygan River traps (June 2). Many of these fish were exhibiting secondary sexual characteristics and their average gonadosomatic index was 18.3 ± 1.0% (n = 20). Water temperatures varied between 11 and 14°C for the Black Mallard River and 12–16°C for Grand Lake Outlet during these tests.

The testing device comprised two 4-m-long channels that were constructed from plywood and mounted, next to each other, on pilings driven into the stream bottom (Fig. 3). Their height above the stream bottom was adjusted daily so

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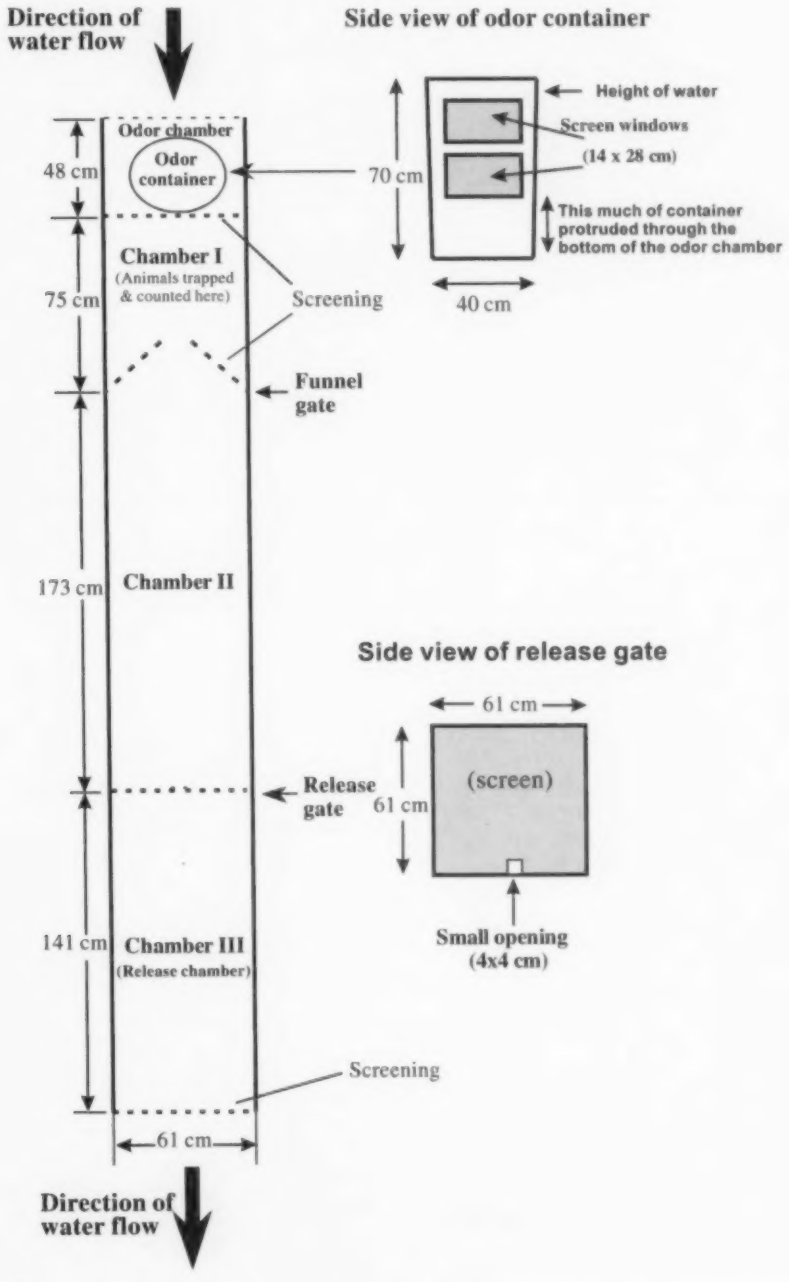
Fig. 3. Schematic representation of one channel of the instream testing device used in Experiment 2. The device comprised two identical channels placed side by side. One channel was then used for control odor administration and the other for larval odor. Dashed lines and cross-hatching represent the screening that separated the chambers within each channel. A side view of the release gate and screened odor container is also shown. The dimensions of the apparatus are indicated. This device was positioned in a stream and operated by placing groups of 10 lamprey into the release chamber (III) and counting how many swam into the upstream chamber (I) within 30 min.



that water depth within each channel was always 30 cm. Each channel had four chambers: a front-end chamber that served to hold the odor source (see below), an upstream chamber (I) that served to trap those animals that made it upstream, a middle chamber (II), and a downstream chamber (III) into which animals were initially released. The gate be-

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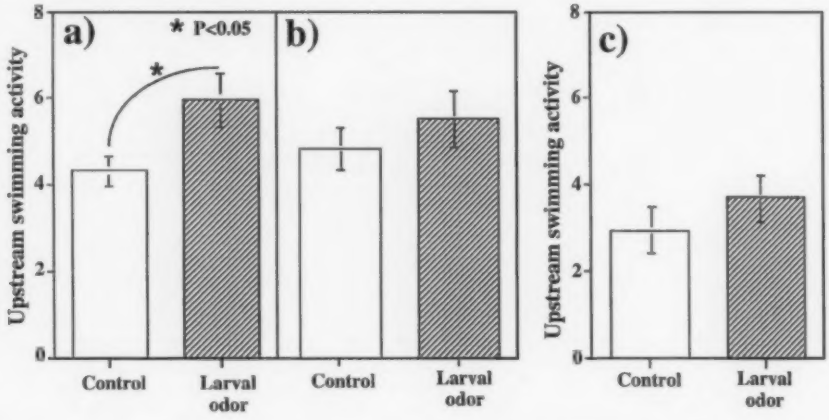
Fig. 4. Results of Experiment 2: upstream swimming activity of migratory sea lamprey tested in a stream. The average number (±SE) of animals caught in chamber I of the channel with the sand control is compared with the number caught in chamber I of the channel with larval odor. Three sets of experiments were run: (*a*) early migrants tested in the early evening (n = 14 groups of 10 animals), (*b*) early migrants tested in the late evening (n = 6 groups of 10 animals), and (*c*) late (sexually maturing) migrants tested in the early evening (n = 24 groups of 10 animals).



tween the release and middle chambers had a small 4 × 4 cm opening, restricting upstream movement to only the most persistent animals. The velocity of the water passing through each channel was adjusted each evening by adding plastic screens with various mesh sizes to the upstream end of each front-end channel until current velocities of 0.12–0.13 $m \cdot s^{-1}$ were obtained. This velocity falls within the range of that reported in lamprey spawning streams (Stier and Kynard 1986; P.W. Sorensen, personal observation).

Larval odor was produced using a captive population of 300 larval sea lamprey that we maintained in a 50-gal (200-L) plastic container (garbage can) filled to a depth of about 10 cm with sand. An identical container with only sand served as the control. Both containers had two screened openings to allow river water to flow through them. During the day, these containers were held in the stream (to permit larvae to feed on floating detritus), but 30 min prior to the start of an evening of testing, they were placed into the top end of the chambers, the control container into one and the larval container into the other. Openings were cut into the bottom of each chamber so that the containers rested on the stream bottoms and their screened openings lined up with the water level. The positions of larval and control containers were switched every fourth trial (typically once a night) to produce a balanced sample design. No larval mortality was noted.

Typically, eight trials were run per night. Tests commenced 1 h after sunset (about 22:00) when a group of 10 adult lamprey was placed into each of the release chambers located at the downstream end of each channel. Dim red lights were used when transferring animals. Thirty minutes later, the lamprey were removed from the channels with dip nets and their positions were carefully noted. The number found in the upstream chambers served as our measure of upstream swimming activity. Experiments conducted in the Black Mallard River ran until 04:00 and were analyzed according to whether they were conducted during the first or second half of the night (before or after 02:00). Experiments conducted in the Grand Lake Outlet ended at 02:00. We ran 6–24 replicates of each test depending on what weather and water flow would allow. Upstream swimming activity was evaluated by comparing the number of animals found in the upper chamber of the control channel with that found in the upper channel of the experimental channel at the end of each set of trials using paired t tests (two-tailed; Instat, Calif.). Sample size was the number of groups tested.

Results

Lamprey caught early in the spawning run and tested in the early evening swam upstream more vigorously when exposed to larval odor than when exposed to the odor of sand alone ($P < 0.05$) (Fig. 4*a*). Interestingly, this response disappeared later in the night (Fig. 4*b*). Sea lamprey captured at the end of their migrational period and relatively mature (June 2–4) also showed no response to larval odor, even when tested in the early evening (Fig. 4*c*).

Experiment 3. Does the odor of larval bile acids influence the activity and distribution of migratory sea lamprey in still well water?

In late 1993, we discovered that larval sea lamprey produce and release two unique bile acids (PS and ACA) and that both of these function as potent olfactory stimulants (Li et al. 1995). Consequently, we became interested in testing whether these compounds might be a key component of the larval pheromone. The primary objective of Experiment 3 was to determine whether bile acids influence sea lamprey behavior in a manner similar to that of larval odor. Because neither PS nor ACA was commercially available, a laboratory experiment that required only small amounts of compounds was needed and a new apparatus using still water and large circular tanks (3 m in diameter) was designed. First, we tested the effects of a mixture of PS and ACA on swimming activity and distribution of migratory adult lamprey. Next, we tested the effects of larval odor on their be-

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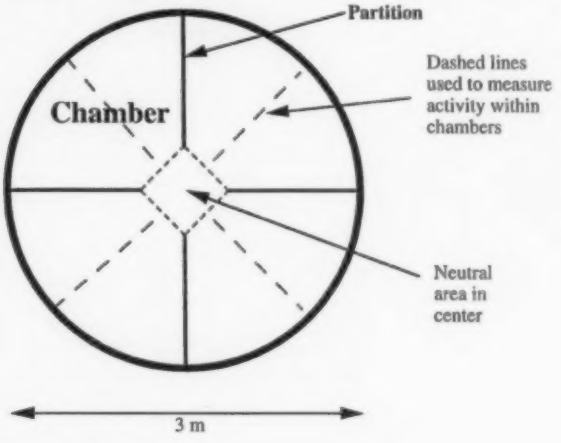
Fig. 5. Schematic representation of the circular test devices used to test the responses of migratory sea lamprey to bile acids and larval odor in still well water in the laboratory (Experiment 3). Two of these devices were constructed, each of which was divided into four chambers by barriers that extended from their periphery to a central area that remained open to permit movement between chambers. A line was drawn down the center of each chamber to evaluate movement rate and across the entrance so that distribution could be monitored. Odor and control solutions were pumped into the center of each chamber. Groups of 12 animals were tested.



havior. This experiment is the first that we know of to test the behavioral responsiveness of a migratory fish to the specific set of bile acids known to be released by its conspecifics.

Materials and methods

Adult sea lamprey were trapped during the first week of their migratory period in the St. Mary's River (Ontario) in early July 1994 and transported to our laboratory (St. Paul, Minn.) in aerated containers. Only females with no obvious secondary sexual characteristics were used. Once in the laboratory, fish were held under a natural photoperiod in flowing, chilled (6°C) well water to inhibit sexual maturation. Two days before being used for a test, groups of 50 animals were warmed to 14°C overnight by slowly increasing tank temperature.

The testing apparatus consisted of two large circular tanks (3 m in diameter) that were each divided into four equal-sized chambers by plywood walls extending from their sides (Fig. 5). A circular open area (50 cm in diameter) in the middle of each tank allowed the lamprey to swim freely between chambers. Lines were drawn down the center of each chamber so that fish movement (line crossings) could be monitored within each. Tanks were illuminated by a combination of ambient lighting (outside windows) and a dim overhead light that was reflected off the ceiling. Each tank was supplied with well water (14 ± 0.5°C) at a flow rate of about 50 $L \cdot min^{-1}$ that was turned off 15 min before experiments started (see below). Water depth was about 14 cm, giving the tanks a volume of 1000 L. Tygon "odorless" tubing (R-B44-3; Fisher, Pittsburgh, Pa.) was fixed to the outside edge of the midline of each chamber for use in odor injection. Electroolfactogram (EOG) recording from the olfactory epithelium of lamprey confirmed that this tubing was without odor to lamprey (P.W. Sorensen, unpublished results). Dye tests showed that when we injected water through these tubes at 50 $mL \cdot min^{-1}$, rapid mixing occurred and there was little mixing between chambers for the first 30 min.

Tests were conducted using groups of 12 animals that were introduced into the tanks the morning before experiments were conducted. Two trials were run each night by employing both tanks; one tank was used "early," starting at dusk and ending by sunset (20:30), and the other was used "late," starting at 21:30 (1 h after sunset to avoid testing during twilight when light levels were changing rapidly). The order in which tanks were tested was reversed each night to eliminate bias. Fish behavior was noted by two observers who were unaware of the identities of the odors being tested and were positioned at opposite ends of each tank and positioned so as not to disturb the lamprey. Each observer noted lamprey behavior in one pair of chambers.

Experimental trials commenced with a 5-min stimulus-habituation period, during which time the control odor (either methanol control or the odor of sand; see below) was pumped into all four chambers of each tank. This was immediately followed (without a change in odor addition) by the pretest, during which time the lamprey were observed. We observed lamprey for three consecutive 10-min pretest periods, each separated by a 10-min break. Then, the flow of control odor was halted to two chambers (selected using a random number table) and test odor was added to them instead. Control odor continued to be added to the other two chambers. Five minutes later, observations recommenced and three 10-min observation test periods, each separated by a 10-min break, were conducted. Both swimming activity and distribution were noted during the pretest and test periods. Swimming activity was quantified as the total number of times animals were seen to cross the lines dividing each chamber; it was noted on a continuous basis. Distribution was quantified as the number of animals counted in each chamber at 1-min intervals. The data from each set of three pretest periods and each set of test periods were later combined by chamber and the data associated with each chamber were totaled to provide values for the test chambers during the pretest and test periods.

In our initial set of experiments, we wished to test lamprey bile acids at concentrations that approximated those found in rivers. We based our design on analyses of lamprey rivers using high-performance liquid chromatography, which found PS present at a concentration of about 5×10^{-10} M (P.W. Sorensen, unpublished data; Polkinghorne 1997). To mimic this scenario, we pumped a mixture of 5×10^{-8} M PS and 5×10^{-6} M cholic acid (CA; the 5β isomer of ACA; Sigma Chemical Co., St. Louis, Mo.) into the test chambers at a rate of 50 $mL \cdot min^{-1} \cdot chamber^{-1}$. Methanol carrier alone (1 $mL \cdot L^{-1}$ [a concentration not detected by EOG recording] at 50 $mL \cdot min^{-1}$) was used as the control for both the pretest and the test periods. If odor was uniformly diluted by the end of the test period (as appeared to be the case), we were testing a concentration of 5×10^{-10} M PS in the final test period. PS was obtained by partially purifying the contents of

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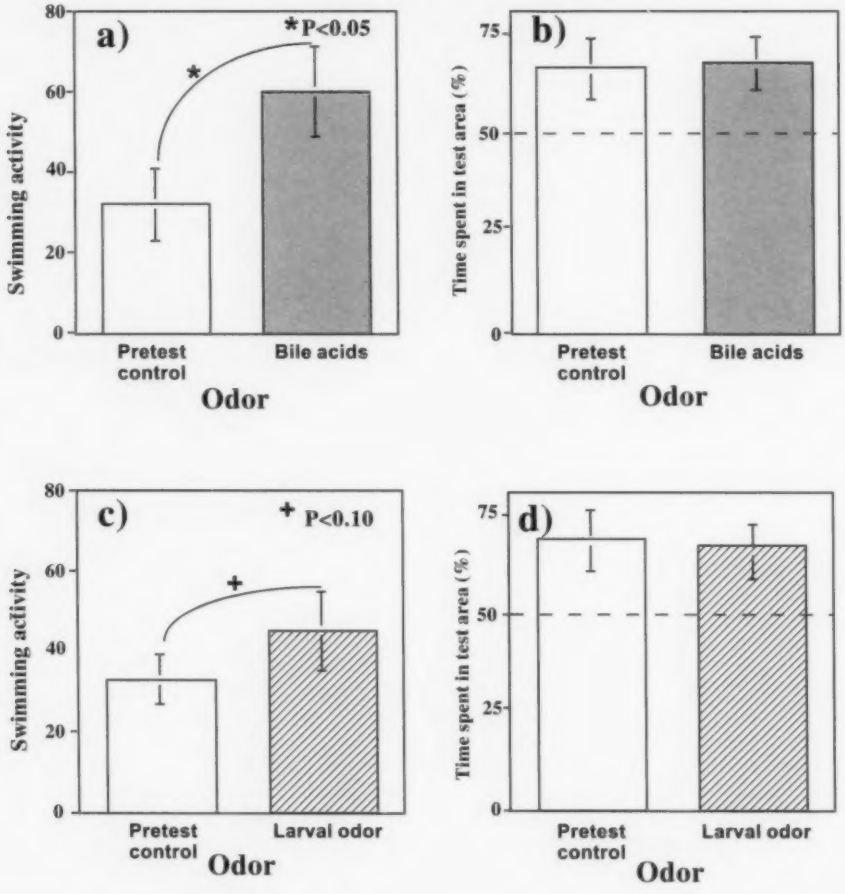
Fig. 6. Results of Experiment 3: behavioral responses of migratory sea lamprey to either a mixture of PS and CA or larval odor. (*a*) Average number (±SE) of line crossings performed by sea lamprey located in test chambers when they were exposed to a methanol control during the pretest period and then to bile acids during the test period (n = 14 groups of 12 animals;. (*b*) average percent time (±SE) spent by sea lamprey in the test chambers when they were exposed to a methanol control during the pretest period and then to bile acids during the test period (n = 14 groups of 12 animals); (*c*) average number (±SE) of line crossings performed by sea lamprey in the test chambers when they were exposed to a sand rinse (control) during the pretest period and then to larval odor during the test period (n = 6 groups of 12 animals); (*d*) average percent time (±SE) spent by sea lamprey in the test chambers when they were exposed to a sand rinse (control) during the pretest period and then to larval odor during the test period (n = 6 groups of 12 animals).



200 larval gallbladders using C18 Sep-Paks columns (Waters Co., Milford, Mass.) and then quantifying it by high-performance liquid chromatography and mass spectrometry (Li et al. 1995). We mixed the PS with CA. CA was used as a substitute for ACA because the latter was not available from commercial sources. This substitution was employed because, although CA is not produced by lamprey, EOG cross-adaptation experiments have shown that CA cross-reacts completely with the ACA olfactory receptor (Li and Sorensen 1997). Because CA is 1000 times less potent than ACA (as measured by EOG; Li and Sorensen 1997), we had to use 1000 times more CA.

In a second set of experiments, we tested the effects of larval odor. For larval odor, we pumped well water at a rate of 100 mL·min^{-1} from a 50-L holding tank that had held 150 larval sea lamprey in washed sand for 24 h. Its control was water from a holding tank lacking larvae. The larvae were fed bakers yeast once every few days when they were not being used for odor production. The second test was only conducted six times, as we were unable to obtain new lamprey.

Data were analyzed in the same way for both sets of experiments. Three types of analysis were performed. First, changes in activity were evaluated by comparing the total number of line crossings noted in the test chambers during the pretest periods with that measured during the test periods. Because the data values were matched across time, comparisons were made using paired t tests (two-tailed; Instat, Calif.). Second, distribution was analyzed by comparing the total number of animals seen in the test chambers during the pretest (control) period with that seen during the corresponding test period. Values were transformed to reflect

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percent total time spent by groups of lamprey in the test chambers (the total number of animals seen in the test chambers for each 30-min period was divided by 360). Paired *t* tests (two-tailed) were also used for this analysis. Finally, "relative" activity was analyzed to verify that any changes seen in the test chambers were attributable to odor and not to time of day (a concern because odor was always added later in the day and lamprey are most active at night). Relative activity was calculated as the percentage of total activity (i.e., all line crossings) seen in each tank that was attributable to the activity occurring in just the test chambers. Relative activity was compared between pretest and test periods for each experiment using paired *t* tests (two-tailed).

Results

Migratory adult sea lamprey increased their swimming activity when exposed to the mixture of conspecific bile acids ($P < 0.05$) (Fig. 6*a*) as well as to larval odor, although the latter increase was not significant ($P = 0.10$) (Fig. 6*c*). Notably, the power of the latter test was limited by sample size ($n = 14$ versus 6 trials). Time of day could not account for these increases in swimming activity because relative activity also increased: 44% of all activity occurred in test chambers prior to bile acid addition versus 56% afterwards ($P < 0.05$, $n = 14$ trials). Similarly, 44% of all activity occurred in test chambers prior to larval odor addition versus 53% afterwards ($n = 6$ trials). Neither the addition of bile acids nor the addition of larval odor altered the distribution of animals (Figs. 6*b* and 6*d*).

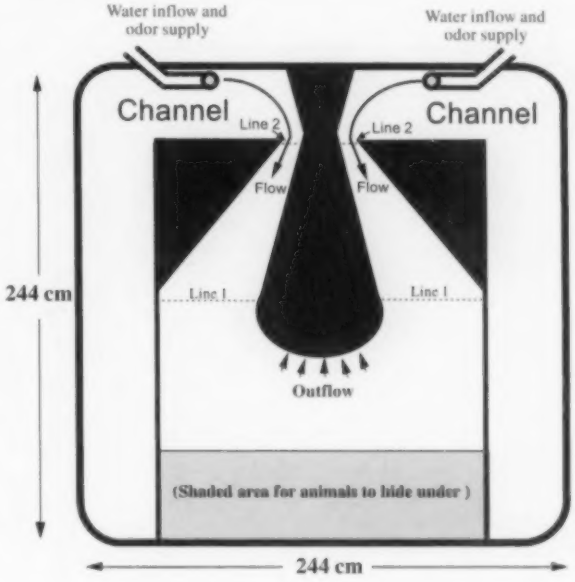
Experiment 4. Does the odor of larval bile acids influence the activity of migratory sea lamprey in flowing well water?

Experiment 3 demonstrated that exposure to bile acids and larval odor elicited increased swimming activity in still water but, surprisingly, did not attract these animals. One possible explanation was that lamprey, like migratory salmon, only exhibit oriented movement into an odor source when it is associated with discernable flow; i.e., attraction occurs through rheotaxis (Kleerekoper 1967; Johnsen and Hasler 1980). Accordingly, we conducted Experiment 4 to test whether migratory adult sea lamprey showed an increased tendency to swim into flowing water when conspecific bile acids were added to it. During the course of this experiment, we varied the rates of flow (and concurrently the velocity) between the test and control chambers because we wanted to assess the effects of odor independent of flow.

Materials and methods

Adult sea lamprey were trapped during the first few weeks of their migratory period in the St. Mary's River in July 1995 and transported to St. Paul where they were held in 1000-L holding tanks supplied with flowing 12°C well water. Immature animals of both sexes were used in these experiments. Two identical testing devices were constructed. Each consisted of a square (244 cm on each side) fiberglass tank with rounded corners into which two Plexiglas barriers were inserted to create two J-shaped channels at one end through which flows were accelerated (Fig. 7). A screened drain was located in the center of each tank so that water

Fig. 7. Schematic representation of the testing device used in Experiment 4 to assay the behavior of adult lamprey exposed to bile acids in flowing well water. Lines refer to marks on the bottom of the maze used to monitor swimming activity. Line 1 denotes the boundary of each channel. Water flowed down each channel, around the J-shaped areas, and then exited via a central drain. Groups of five animals were released from the terminal holding chamber and their behavior observed while control and test odors were administered down each arm via the inflowing water. Water flows down each channel were also systematically altered (see text).



flowed from the opening of each channel down the center without mixing. Opposite the drain, an overhead screen was placed to provide an area of darkness for lamprey during the day. One set of lines (line 1) was drawn at the front of each chamber opening so that we could determine when animals entered the flows. Another set of lines (line 2) was drawn at the back of each chamber to measure activity within chambers. Inflowing well water (13°C) came from a pipe in the curved section of each channel where depth was maintained at 15 cm. Each inflow line had a needle valve so that flow could be quickly and precisely adjusted to levels previously set with the aid of a flowmeter. Odor solutions were added to the chamber via the incoming water using Tygon odorless tubing (R-B44-3). Dye tests indicated that odor was immediately mixed and that odor separation between the chambers was nearly perfect. Tanks were illuminated by an overhead infrared 200-W light, the spectrum of which (780–900 nm) fell outside the visual range of the lamprey retina (690 nm; D. Burkhardt, University of Minnesota, St. Paul, Minn., unpublished results). An infrared-sensitive monochrome camera was mounted above each tank and the images stored on a video recorder for analysis at a later time.

Groups of five animals were introduced into each apparatus the afternoon prior to each experiment when the water flowing down each set of channels was also adjusted to a ve-

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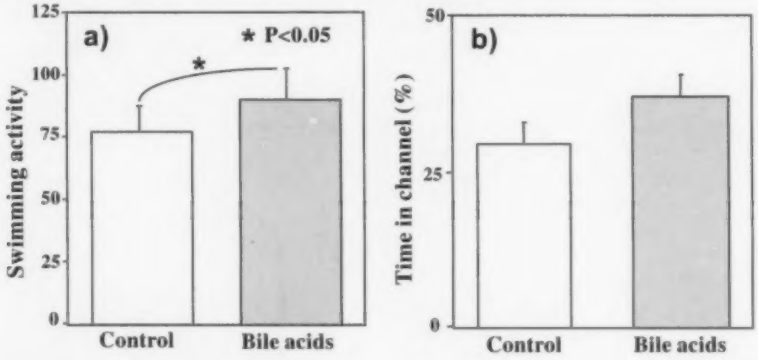
Fig. 8. Results of Experiment 4: behavioral responses of migratory sea lamprey offered a choice of flowing well water containing either a methanol control or a mixture of lamprey bile acids (PS and ACA). (*a*) Average total (line 1 + line 2) number (±SE) of line crossings by the groups of sea lamprey in the channel with the methanol control and the channel with bile acids during both test periods (n = 8 groups of 5 animals); (*b*) average percent time (±SE) that sea lamprey were present in the control and bile acid channels (n = 8 groups of 5 animals). The score did not add up to 100%, as animals were often in the central area.



locity of about 0.10 m·s^{-1} (similar to that used in Experiment 2). Two experiments were run per night, one per tank. The first experiment started after sunset (22:00) and the second at about 24:00. At the start of each experiment, current velocities were readjusted so that the entrance of one channel had a velocity of 0.06 m·s^{-1} (flow of 19 L·min^{-1}) and the other a velocity 0.15 m·s^{-1} (flow of 47 L·min^{-1}). Control (methanol carrier) solution was then introduced into the inflow of one channel and test odor (conspecific bile acids; see below) into the other using a two-channel peristaltic pump. The first test period lasted 15 min, after which there was a 5-min break, during which time the flow rates were altered so that the channel that had the slow rate now had the fast one and vice versa. A second 15-min test period then commenced. Odor addition was not altered during the break. The channels used for each odor were reversed daily to create a balanced design.

For a test odor, we used a mixture of 10^{-6} M PS and 10^{-6} M ACA dissolved in methanol–water (1 mL·L^{-1}) pumped at a rate (20 mL·min^{-1}) designed to produce a concentration (assuming complete mixing within the channel) of about 5 × 10^{-10} M when added to water flowing at the slower of the two rates. This concentration was the same as that used in Experiment 3 and decreased by a factor of about 2.5 when the channel flow rate was increased. PS was obtained by extracting and purifying lamprey gallbladders; ACA was obtained by custom synthesis (Toronto Research Chemicals, Toronto, Ont.). For a control odor, we used methanol at a concentration of 1 mL·L^{-1}. Both the control odor and the test odor were pumped in a pulsatile manner (15 s on, 15 s off), thereby saving odor and simulating the fragmentation that aquatic odor plumes are naturally subjected to and that often appears to be important to orientation (Atema 1995).

Behavior was analyzed after the conclusion of each experiment by viewing video tapes in slow motion. Upstream swimming activity was quantified by counting the number of times fish crossed both lines in each channel, while distribution was determined by counting the number of animals located in each channel (upstream of line 1) at 30-s intervals. This number was then converted to percent time by dividing it by 300, the score that would have been recorded had all five animals been in one channel for all 60 time intervals. Two analyses were performed using these values. First, we examined the effects of current velocity on upstream swimming by comparing the activity (line crossings) observed within the bile acid channels during the test period when they had low flow with that of the corresponding test period when they had high flow. Next, because current velocity did not appear to affect behavior, we combined the two test trials within each experiment and compared the swimming activity seen in the control odor with that seen in conspecific bile acid odor by paired t tests (two-tailed; Instat, Calif.). The number of animals seen within chambers was also compared by paired t tests (two-tailed).

Results

On average, lamprey swam upstream approximately 20% more actively when exposed to a mixture of PS and ACA than when exposed to the methanol control ($P < 0.05$) (Fig. 8*a*). The strength of this response was not influenced by relative current velocity or the small differences in bile acid concentration associated with it, as lamprey exhibited as much activity in the odor channel when it had a slow flow as when it had a fast flow (84.0 ± 12.5 versus 82.1 ± 11.7 line crossings). Interestingly, bile acid odor did not appear to be attractive (Fig. 8*b*). A review of the video tapes suggested that this was attributable to the fact that few lamprey swam significant distances into the channels; bile acids apparently caused animals to swim up to the odor source (crossing line 1) but did not often cause them to enter it fully.

General discussion

Taken together, the results of our studies lend support to the hypothesis that unique bile acids released by larval sea lamprey attract migratory adults into rivers to spawn. It is not difficult to imagine how these unique chemical cues might have assumed these functions over evolutionary time, for they represent universal and direct indicators of habitat suitability. Our results are notable because all four of our experiments describe similar, relevant behavioral responses to low concentrations of larval odor and the bile acids that it

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contains. Our findings fully support earlier studies of adult behavioral responsiveness to larval odor (Teeter 1980), the observation that adult lamprey capture rates drop in rivers when their larval populations (odor) are reduced (Moore and Schleen 1980), and our finding that the olfactory system of this species possesses extremely specific and sensitive receptors for PS and ACA (Li et al. 1995; Li and Sorensen 1997). In summary, we feel confident that we have demonstrated that larval odor and the two bile acids that it contains function as a pheromone that attracts adult sea lamprey. A concerted effort to elucidate the natural biological function, potency, and complete chemical identity of this cue is now warranted.

Water velocity appears to play a role in how lamprey orient to the larval pheromone, but its exact role is not yet clear. Thus, although larval odor was attractive when added to slowly flowing well water (Experiment 1), it did not attract lamprey when tested in still well water, although it did stimulate their swimming activity (Experiment 3). It is interesting to speculate that responsiveness to migratory pheromones reflects some kind of odor-mediated rheotactic response or enhanced swimming into a current (Smith 1985). If true, however, this behavioral mechanism is likely different from that described for salmon, which only swim actively when exposed to flowing home stream odor (Johnsen and Hasler 1980). That lamprey oriented in the absence of a differential flow (Experiment 1) and that their swimming activity was not influenced by the relative strength of the current into which pheromonal odors were added (Experiment 4) also suggests that lamprey use a chemoorientation mechanism that is different from that envisaged by Kleerekoper (1967).

Although it is difficult to speculate as to what the precise chemoorientation mechanism employed by lamprey might be, it is interesting that it appears to be effective in very slow currents such as those used in Experiment 1 (10 cm·min^{-1}). This extreme sensitivity to current makes ecological sense, for when migratory adult lamprey first enter stream mouths, they likely encounter stream/larval odor as a slow-flowing surface layer running over the colder lake (ocean) waters. The fact that lamprey are also poor swimmers and have but a single nose (which precludes simultaneous comparative odor sampling) would appear to make the challenge of locating such an odor source particularly great. Notably, lamprey do not need to precisely locate particular streams like salmon do but only need to find "suitable" streams. How efficient lamprey are at locating suitable spawning streams is unknown at this time, but presumably this trait is actively selected for and thus may be relatively sophisticated. For instance, it is quite possible that riverine odor cues other than bile acids may also be involved in locating streams; indeed, the relatively weak response of lamprey to bile acids alone in Experiment 4 would seem to suggest this possibility. Similarly, Applegate (1950) suggested that temperature has a role in stream location. Experiments comparing the behavioral responses of captive and wild lamprey to natural river waters to which bile acids have been added (or subtracted) are now needed to determine the precise role and importance of conspecific bile acids in stream finding.

Although our study has its limitations, we believe it to be one of the clearest demonstrations to date of a migratory pheromone used by a fish. Previous studies of the effects of conspecific odors on fish migration (e.g., Nordeng 1977; Selset and Døving 1980; Foster 1985; Olsén 1987; Courtenay et al. 1997) have employed concentrated washings of conspecifics and (or) their fecal material, the chemical composition of which was not known. In contrast, we have demonstrated that adult lamprey located in a stream will increase their upstream swimming activity in the presence of dilute larval water (Experiment 2), which we also know to contain two unique bile acids (Li et al. 1995; Polkinghorne 1997). Similarly, our study appears to represent the most direct evidence collected to date in support of Døving et al.'s (1980) hypothesis that conspecific bile acids function as migrational pheromones in fishes. We tested those bile acids that we knew to be produced, released, and detected by lamprey and used concentrations and ratios of these compounds that we have measured in larval release waters and river water (Li et al. 1995; Polkinghorne 1997; P.W. Sorensen, unpublished data). Previous tests of bile acid function in teleost fishes (e.g., European eel (*Anguilla anguilla*) (Sola and Tosi 1993) and Arctic char (*Salvelinus alpinus*) (Jones and Hara 1985)) have not had the luxury of being able to perform biochemical analyses of bile acids released by these species. The use of appropriate bile acid mixtures in testing regimes may be important because we now know that different fish produce and release different mixtures of bile acids (Hoshita 1985; Polkinghorne 1997) and that the olfactory epithelia of these fish have separate and independent olfactory receptors for different bile acids (Li and Sorensen 1997; P.W. Sorensen, unpublished data). Notably, the specificity of most insect pheromones does not lie in the identity(ies) of the specific compounds employed but rather in their precise ratios (Sorensen et al. 1998). The possibility that migratory pheromones of fish also comprise specific mixtures of bile acids warrants consideration.

Our finding that responsiveness to conspecific odor is greatest in the early evening suggests that the larval pheromone plays an active role in the migration of wild lamprey that show the same activity pattern (Skidmore 1959; Stier and Kynard 1986). Similarly, captive lamprey have been shown to possess a strong circadian rhythm in spontaneous activity, suggesting that it underlies the daily pattern in odor-mediated migratory activity that we observed (Morita et al. 1992). Also interesting is our observation that migratory lamprey come to ignore the odor of larvae and develop a preference for mature conspecifics as they themselves mature. This makes ecological sense, for once a lamprey has entered a river, larval cues may be of little importance and it must find mates within a 2- to 4-week period before it dies (Applegate 1950). An understanding of this physiological mechanism is of practical significance, for it suggests that if sea lamprey migration could be delayed until these animals were mature, they might be unable to locate spawning streams. Additionally, it suggests that if sexually mature sterile males are to be used in lamprey control (as proposed), they should not be released far downstream where only larval odor (and not sex pheromones) might be evident. Our finding that sexually mature sea lamprey produce sex pheromones that attract adults confirms earlier studies (Teeter 1980; Bjerselius et al. 1995).

In summary, we have now shown that migratory adult sea

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lamprey employ a larval pheromone which stimulates swimming activity and when coupled with flow functions as an attractant. Although it seems reasonable that this cue helps guide lamprey into spawning rivers from lakes (and oceans), it has yet to be directly determined how important this cue is or how it functions. Further, although the parallels that we describe between behavioral responses to "whole" larval odor and its bile acids suggest that bile acids are an important part of the larval pheromone, we have yet to ascertain whether bile acids comprise the entire pheromone or how this odor complements the odor of natural river water. Nevertheless, we are optimistic that once the actions and identity of this pheromone are fully understood, it will have potential for use as an attractant in lamprey control. For example, the pheromone might be metered into existing traps to enhance capture rates or added to streams that lack suitable spawning habitat, thereby causing lamprey to waste their reproductive effort. Such an environmentally friendly and potentially inexpensive tool would be a welcome addition to a lamprey control strategy.

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References

Applegate, V.C. 1950. Natural history of the sea lamprey (*Petromyzon marinus*) in Michigan. U.S. Fish Wildl. Serv. Spec. Sci. Rep. Fish. Serv. 55.

Atema, J. 1995. Chemical signals in the marine environment: dispersal, detection, and temporal signal analysis. Proc. Natl. Acad. Sci. U.S.A. **92**: 62–66.

Bergstedt, R.A., and Seelye, J.G. 1995. Evidence for lack of homing by sea lamprey. Trans. Am. Fish. Soc. **124**: 235–239.

Bjerselius, B., Li, W., and Sorensen, P.W. 1995. Spermiated male sea lamprey release a potent sex pheromone. *In* Proceedings of the Fifth International Symposium on the Reproductive Physiology of Fish. *Edited by* F.W. Goetz and P. Thomas. Fishsymp 95, The University of Texas at Austin, Austin, Tex. p. 271.

Courtenay, S.C., Quinn, T.P., Dupuis, H.M.C., Groot, C., and Larkin, P.A. 1997. Factors affecting the recognition of population-specific odours by juvenile coho salmon. J. Fish Biol. **50**: 1042–1060.

Døving, K.B., Selset, R., and Thommesen, G. 1980. Olfactory sensitivity to bile acids in salmonid fishes. Acta Physiol. Scand. **108**: 123–131.

Foster, N.R. 1985. Lake trout reproductive behavior: influence of chemosensory cues from young-of-the-year by-products. Trans. Am. Fish. Soc. **114**: 794–803.

Hardisty, M.W., and Potter, I.C. 1971. The general biology of adult lampreys. *In* The biology of lampreys. Vol. 1. *Edited by* M.W. Hardisty and I.C. Potter. Academic Press, New York. pp. 127–206.

Hoshita, T. 1985. Bile alcohols and primitive bile acids. *In* Sterols and bile acids. Vol. 12. *Edited by* H. Danielsson and J. Sovall. Elsevier Press, Amsterdam, The Netherlands. pp. 279–301.

Johnsen, P.B., and Hasler, A.D. 1980. The use of chemical cues in the upstream migration of coho salmon, *Oncorhynchus kisutch* Walbaum. J. Fish Biol. **17**: 67–73.

Jones, K.A., and Hara, T.J. 1985. Behavioral responses of fishes to chemical cues: results from a new bioassay. J. Fish Biol. **27**: 495–504.

Karlson, P., and Lüscher, M. 1959. "Pheromones": a new term for a class of biologically active substances. Nature (Lond.), **183**: 55–56.

Kleerekoper, H. 1967. Some aspects of olfaction in fishes, with special references to orientation. Am. Zool. **7**: 385–395.

Li, W., and Sorensen, P.W. 1997. Highly independent olfactory receptor sites for naturally occurring bile acids in the sea lamprey, *Petromyzon marinus*. J. Comp. Physiol. A, **180**: 429–438.

Li, W., Sorensen, P.W., and Gallaher, D.G. 1995. The olfactory system of the migratory sea lamprey (*Petromyzon marinus*) is specifically and acutely sensitive to unique bile acids released by conspecific larvae. J. Gen. Physiol. **105**: 569–587.

Lisowski, J.J., Bushnell, P.G., and Teeter, J.H. 1986. A two-choice water recirculation tank for assessing chemosensory preferences of landlocked sea lampreys. Prog. Fish-Cult. **48**: 64–67.

Moore, H.H., and Schleen, L.P. 1980. Changes in spawning runs of sea lamprey (*Petromyzon marinus*) in selected streams of Lake Superior after chemical control. Can. J. Fish. Aquat. Sci. **37**: 1851–1860.

Morita, Y., Tabata, M., Uchida, K., and Samajima, M. 1992. Pineal-dependent locomotor activity of lamprey, *Lampetra japonica*, measured in relation to LD cycle and circadian rhythmicity. J. Comp. Physiol. A, **171**: 555–562.

Morman, R.H., Cuddy, D.W., and Rugen, P.C. 1980. Factors influencing the distribution of sea lamprey (*Petromyzon marinus*) in the Great Lakes. Can. J. Fish. Aquat. Sci. **37**: 1811–1826.

Nordeng, H. 1977. A pheromone hypothesis for homeward migration in anadromous salmonids. Oikos, **28**: 155–159.

Olsén, K.H. 1987. Chemoattraction of juvenile Arctic charr *Salvelinus alpinus* (L.) to water scented by conspecific intestinal content and urine. Comp. Biochem. Physiol. A, Comp. Physiol. **87**: 641–643.

Polkinghorne, C.N. 1997. Determining whether bile acids released by larval sea lamprey and other fishes may be functioning as species-specific migratory cues. M.S. thesis, University of Minnesota, St. Paul, Minn.

Purvis, H.A. 1980. Effects of temperature on metamorphosis and the age and length at metamorphosis in sea lamprey (*Petromyzon marinus*) in the Great Lakes. Can. J. Fish. Aquat. Sci. **37**: 1827–1834.

Selset, R., and Døving, K.B. 1980. Behavior of mature anadromous char (*Salmo alpinus* L.) towards odorants produced by smolts of their own population. Acta Physiol. Scand. **108**: 113–122.

Skidmore, J.F. 1959. Biology of spawning-run sea lampreys (*Petromyzon marinus*) in the Pancake River, Ontario. M.S. thesis, University of Western Ontario, London, Ont.

Smith, B.R., and Tibbles, J.J. 1980. Sea lamprey (*Petromyzon marinus*) in Lakes Huron, Michigan, and Superior: history of in-

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vasion and control, 1936–1978. Can. J. Fish. Aquat. Sci. **37**: 1780–1801.

Smith, R.J.F. 1985. The control of fish migration. Springer-Verlag, Berlin.

Sola, C., and Tosi, L. 1993. Bile salts and taurine as chemical stimuli for glass eels, *Anguilla anguilla*: a behavioral study. Environ. Biol. Fishes, **37**: 197–204.

Sorensen, P.W., Christensen, T.A., and Stacey, N.E. 1998. Discrimination of pheromonal cues in fish: emerging parallels with insects. Curr. Opin. Neurobiol. **8**: 458–467.

Stacey, N.E., and Sorensen, P.W. 1991. Function and evolution of fish hormonal pheromones. *In* Biochemistry and molecular biology of fishes. Vol. 1. *Edited by* P.W. Hochachka and T.P. Mommsen. Elsevier, Amsterdam, The Netherlands. pp. 109–135.

Stier, K., and Kynard, B. 1986. Movement of sea-run sea lampreys, *Petromyzon marinus*, during the spawning migration in the Connecticut River. Fish. Bull. U.S. **84**: 749–753.

Teeter, J. 1980. Pheromone communication in sea lampreys (*Petromyzon marinus*): implications for population management. Can. J. Fish. Aquat. Sci. **37**: 2123–2132.

Torblaa, R.L., and Westman, R.W. 1980. Ecological impacts of lampricide treatments on sea lamprey (*Petromyzon marinus*) ammocetes and metamorphosed individuals. Can. J. Fish. Aquat. Sci. **37**: 1835–1850.

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Effect of introduced piscivores on native minnow communities in Adirondack lakes

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Abstract: We compared the minnow assemblages of Adirondack lakes with top piscivores with those of lakes having similar physiochemical and biotic characteristics but no top piscivores using a subset of data collected from 1984 to 87 by the Adirondack Lake Survey Corporation. Native minnow richness in lakes with top piscivores was about one third that of lakes without piscivores, with piscivore assemblages dominated by introduced species such as northern pike (*Esox lucius*), largemouth bass (*Micropterus salmoides*), and smallmouth bass (*Micropterus dolomieu*). There was strong evidence that at least four minnow species, including creek chub (*Semotilus atromaculatus*), northern redbelly dace (*Phoxinus eos*), blacknose dace (*Rhinichthys atratulus*), and common shiner (*Luxilus cornutus*), were less likely to occur in lakes with piscivores; for a fifth species (pearl dace (*Margariscus margarita*)), the evidence is suggestive but not as strong. Of 13 minnow species, only for two nonnative species (bluntnose minnow (*Pimephales notatus*) and golden shiner (*Notemigonus crysoleucas*)) was there strong evidence that their occurrence was unaffected by the presence of introduced piscivores. These results add to the growing body of evidence that the introduction of top piscivores to small temperate lakes puts native minnow communities at high extinction risk.

Résumé : Nous avons comparé les assemblages de cyprinidés de lacs des Adirondacks renfermant des piscivores supérieurs à ceux de lacs ayant des caractéristiques physicochimiques et biotiques similaires mais dépourvus de piscivores supérieurs en utilisant un sous-ensemble de données recueillies de 1984 à 1987 par l'Adirondack Lake Survey Corporation. La diversité de cyprinidés indigènes dans les lacs renfermant des piscivores supérieurs était environ trois fois inférieure à celle observée dans les lacs dépourvus de piscivores supérieurs. Les assemblages de piscivores étaient dominés par des espèces introduites comme le grand brochet, l'achigan à grande bouche et l'achigan à petite bouche. Les données montrent assez clairement que la probabilité de présence dans les lacs avec piscivores est plus faible pour au moins quatre espèces de cyprinidés, soit le mulet à cornes, le ventre rouge du nord, le naseux noir et le méné à nageoires rouges; dans le cas d'une cinquième espèce, le mulet perlé, les données vont aussi dans ce sens mais sont moins concluantes. Parmi treize espèces de cyprinidés, seules deux espèces introduites (le ventre-pourri et la chatte de l'est) ne seraient vraisemblablement pas affectées par la présence de piscivores introduits. Ces résultats vont dans le sens des résultats de plus en plus nombreux qui tendent à montrer que l'introduction de piscivores supérieurs dans les petits lacs tempérés expose les assemblages indigènes de cyprinidés à un risque d'extinction élevé.

[Traduit par la Rédaction]

Introduction

Minnows (Cyprinidae) are the most speciose of freshwater fish families and one of the most threatened. Several studies (e.g., Angermeier 1995; Scott and Hall 1997; Whittier et al. 1997) have documented local or regional minnow extirpations, presumably as a consequence of anthropogenic stress. These and other studies suggest that minnows are sensitive to a wide range of anthropogenic disturbances and, as such, may be useful indicators of the integrity of aquatic ecosystems (Hughes and Noss 1992; Scott and Hall 1997; Harig and Bain 1998).

Sensitivity to anthropogenic stress has been inferred primarily from changes in minnow assemblages along stress gradients. Sensitivity to introduced piscivores has been inferred from the negative correlation, at local or regional scales, between the intensity of piscivory (presence–absence or abundance of introduced piscivores) and minnow species richness or abundance (Tonn and Magnuson 1982; Robinson and Tonn 1989; Chapleau et al. 1997). Similarly, several studies have shown reduced minnow species richness associated with low pH (Haines and Baker 1986; Christensen et al. 1990; Matuszek et al. 1990), a finding consistent with the reduced minnow richness of naturally acidic lakes (Rahel and Magnuson 1983).

Inferring sensitivity of minnow assemblages to particular stressors from changes along a stress gradient is problematic because stress gradients often covary among themselves as well as with other physical and biogeographic factors influencing minnow communities. For example, several studies

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have reported positive correlations between lake area and pH (Rahel 1986; Rago and Weiner 1986; Matuszek and Beggs 1988), so that estimating the effect of the latter requires that the effects of the former be statistically controlled. Whittier et al. (1997) showed that northeastern U.S. lakes with extensive shoreline disturbance also tended to have introduced littoral piscivores, an example of spatial covariance in stress gradients. In these instances, estimation of the effect of one stressor requires that others be controlled in some manner.

Here, we test the hypothesis that the introduction of top piscivores results in local minnow extirpation using the extensive Adirondack Lake Survey Corporation (ALSC) database (Baker et al. 1990). To estimate the effect of top piscivores, we compare each lake having piscivores with a lake having no piscivores but similar physiochemical and biotic characteristics. In this manner, we reduce the confounding effects of these other factors without having to fit response surfaces in a high-dimensional space — an exercise fraught with all manner of statistical and methodological difficulties.

Methods

Database and fish assemblage description

The data were obtained from the extensive database assembled by the ALSC in its 1984–1987 survey of the physiochemical and biotic attributes of 1469 lakes in the Adirondack region of northern New York State. This survey was designed to evaluate the effects of acid precipitation on the biotic and abiotic characteristics of Adirondack lakes. A full description of the sampling design and field methods as well as maps of the study region are given in Baker et al. (1990). Specific requests for data should be submitted in writing with a research proposal to the ALSC (New York State Department of Environmental Conservation (NYSDEC)), Route 86, RayBrook, NY 12977, U.S.A.

In the present analysis, we consider only those lakes that had at least one fish species present at the time of the survey and that had no known history of stocking of any species by state or federal agencies, fish and game clubs, or the like. Stocking may influence the distribution of different species in Adirondack lakes (Christensen et al. 1990), and we will report on the influence of stocking on Adirondack minnow communities elsewhere. By excluding lakes that have documented stocking histories, we established a baseline effect of top piscivores in the absence of stocking, which can then be compared with the joint effects of stocking (of nonnative piscivores or other species) and piscivore introductions in lakes where both have occurred. Our final subset included 506 lakes.

Five species of top piscivores were present in the sample of lakes used here: northern pike (*Esox lucius*), smallmouth bass (*Micropterus dolomieu*), largemouth bass (*Micropterus salmoides*), chain pickerel (*Esox niger*), and walleye (*Stizostedion vitreum*). There is good evidence that the first three are not native to the region, having been introduced sometime after the mid-1800s (Gallagher and Baker 1990). Whittier et al. (1997) also considered the latter two to be nonnative to the region. All five species are littoral predators, with the possible exception of walleye, which is primarily a pelagic species.

Thirteen minnow species occurred in the lakes included in the study: golden shiner (*Notemigonus crysoleucas*), creek chub (*Semotilus atromaculatus*), northern redbelly dace (*Phoxinus eos*), pearl dace (*Margariscus margarita*), blacknose dace (*Rhinichthys atratulus*), common shiner (*Luxilus cornutus*), bluntnose minnow (*Pimephales notatus*), lake chub (*Couesius plumbeus*), fathead minnow (*Pimephales promelas*), finescale dace (*Phoxinus neogaeus*), longnose dace (*Rhinichthys cataractae*), cutlips minnow (*Exoglossum maxillingua*), and bridle shiner (*Notropis bifrenatus*). Banded killifish (*Fundulus diaphanus*) (Cyprinodontidae) was also present. Most of the aforementioned species are native to at least part of the Adirondack region, with three exceptions. Golden shiner is not native but is now widely distributed, in part because of its popularity among sport fishers as a baitfish (Gallagher and Baker 1990). Bluntnose minnow is another popular baitfish that is not native to the Adirondacks (NYSDEC 1927–1939) but is now quite common, and banded killifish is probably not native to at least the upland portions of the Adirondacks (Whittier et al. 1997). Because these species are not native to the region, we excluded them from our analysis of native species richness. However, we retained them in our analysis of the effects of top piscivores on individual species.

Analytical approach

Several previous studies have compared fish communities in lakes with and without piscivores (e.g., Tonn and Magnuson 1982; Persson 1997; Whittier et al. 1997) while attempting to control for other differences between the two groups by fitting models that take into account these variables. However, when the number of independent variables is relatively large compared with the total number of observations and (or) there exist linear relationships among independent variables (multicollinearity), deciding on the most appropriate model is often difficult (Draper and Smith 1981). This problem is compounded when relationships between independent variables are nonlinear. In such cases, application of standard methods such as principal components regression does not generally result in significant dimensionality reduction. This in turn translates into relatively high model uncertainty, so that the extent to which other variables actually have been controlled may be considerably less than we think.

Moreover, any model fitting exercise assumes that the partial derivatives of the dependent variables are at least piecewise continuous functions. But for some variables, this may not be true. In the case of the ALSC data, there appears to be a polygonal relationship between minnow richness and pH (Fig. 1*a*). Consequently, the map between richness and pH is one-to-many (i.e., nonfunctional). Similar relationships exist for other variables (Fig. 1) and are not uncommon in this data set or elsewhere. For example, Whittier et al. (1997) documented a relationship between native minnow richness and watershed disturbance that appears to be of this form. It is, of course, possible that such nonfunctional bivariate relationships (Fig. 1) are a consequence of the projection of points in a higher-dimensional space onto a two-dimensional surface, so that while the marginal relationships are not functions, the true partial relationships are. Unfortunately, determining whether this is the case is difficult, not least because it requires that we know both the dimensionality and the geometry of the putative higher-dimensional space.

One proposed method for dealing with bivariate polygonal relationships involves estimating the relationship between the upper (or lower) limit(s) of the dependent variable and the independent variable in question. This relationship is a function that can be estimated using standard statistical techniques (Terrell et al. 1996; Scharf et al. 1998). But this method overcomes the problem by changing the problem, i.e., by changing the dependent variable from the variable itself to its upper or lower limit. It is therefore of limited use when our interest is in the variable itself and we wish to investigate the joint effects of several independent variables that may have either functional or nonfunctional relationships with the dependent variable.

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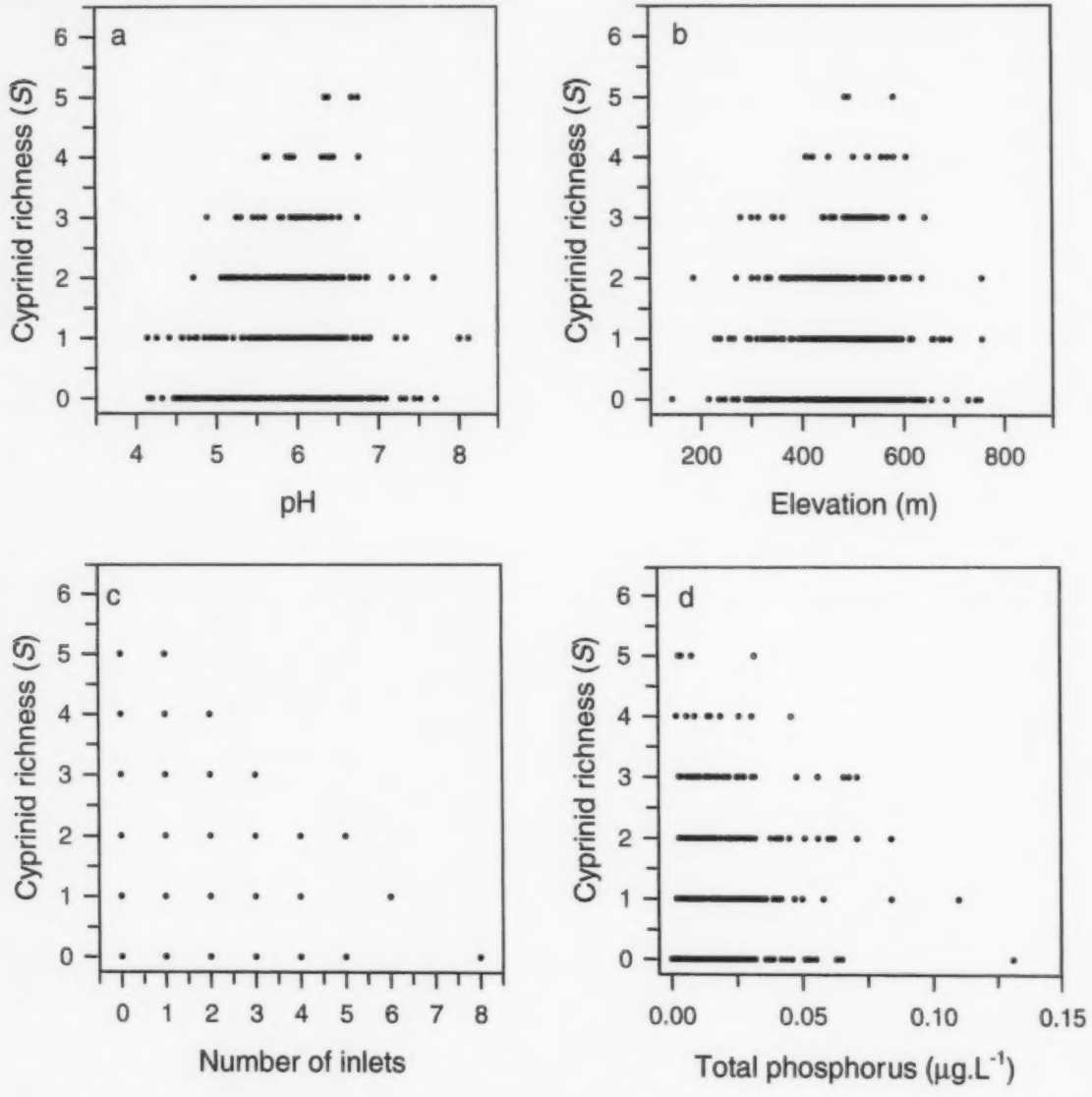
Fig. 1. Relationship between minnow richness S and (*a*) pH, (*b*) elevation, (*c*) the number of stream inlets to the lake, and (*d*) total phosphorus concentrations in Adirondack lakes. All of these bivariate relationships are polygonal rather than functional. See text for further details.



For these reasons, we abandoned statistical control via model fitting and instead attempted to pair each lake with piscivores ("piscivore" (P) lakes) with a lake with no piscivores ("non-piscivore" (NP) lakes) but similar abiotic and biotic characteristics as defined by a set of variables $\mathbf{X} = \{X_1, X_2, ..., X_n\}$. For each P lake, the best NP analog is one having the same values for these variables, such that the "distance" between members of the pair in the space defined by $\mathbf{X}$ is zero. Thus, the NP member of the pair is one that satisfies some neighborhood criterion δ relative to the P lake in question. Clearly, if δ is very small, then few P lakes will have NP analogs, i.e., sample size is small. Hence, while the most stringent control is obtained when δ is small, against this must be weighed the fact that at small δ, statistical power is low.

We define δ based on the Mahalanobis distance D^2. Consider a P lake j. Let $x_{1j}, x_{2j}, ..., x_{nj}$ be the standardized values of variables $X_1, X_2, ..., X_n$ (lake area, pH, etc.) for this lake and let $x^*_{1k}, x^*_{2k}, ..., x^*_{nk}$ be the corresponding values for the kth lake in the set of NP lakes. We then have

$$(1) \qquad D^2_{jk} = \sum_{r=1}^{n}\sum_{s=1}^{n} (x_{rj} - x^*_{rk})\, v_{rs} (x_{sj} - x^*_{sk})$$

where v_{rs} is the element in the rth row and sth column of $\mathbf{V}^{-1}$, with $\mathbf{V}$ the sample covariance matrix. Thus, for every P lake, we can define a neighborhood $\delta = D^2_{\max}$ and test every lake in the NP sample to see whether the latter lies within this neighborhood. This distance measure takes into account differences in scale between the

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various *X*s by using scaled values and correlations between different *X*s by weighting by the inverse of the covariance matrix.

In brief, our analytic procedure was as follows. We paired each P lake in the sample with one or more NP lakes based on the maximum allowable Mahalanobis distance D^2_{max} between the two, calculated from a defined set of control variables (see below). This ensured that each P lake was paired with one or more NP lake(s) having similar values of the control variables, with control stringency determined by D^2_{max} (the smaller the D^2, the greater the control). We then used the set of P–NP pairs to calculate the average pairwise difference in minnow richness. This value was compared with the distribution (over 1000 Monte Carlo trials) of average pairwise differences obtained from randomly assigning each member of a pair to either the P or the NP category. From the resulting (randomized) distribution, we were able to estimate the probability of obtaining the observed average pairwise difference under the null hypothesis that average minnow richness is not reduced in NP lakes. For a full description of the procedure, see the Appendix.

To assess the relationship between minnow community composition and the presence of top piscivores, we used the same approach, scoring 0 for a lake where the species was absent and 1 where it was present. We then calculated the average pairwise difference in scores $\bar{\Delta}$ between P and NP pairs. For any selection of P–NP pairs, the observed average difference $\bar{\Delta}$ and its cumulative distribution $F(\bar{\Delta})$ were obtained from the randomization procedure. Based on F, we calculated the probability p of obtaining an average score at least as large as the observed $\bar{\Delta}$ under the (one-tailed) null hypothesis that the likelihood of the species in question being present is not reduced by the presence of piscivores. Reported p values are Bonferonni corrected for multiple tests of the same hypothesis, one for each species.

Selection of control variables

The ALSC database includes over 50 physiochemical and biotic variables (Baker and Gallagher 1990) in six general classes (Baker and Gallagher 1990): (*i*) acidification response (e.g., lake pH, acid-neutralizing capacity, dissolved organic carbon), (*ii*) habitat suitability (e.g., maximum depth, minimum dissolved oxygen, maximum temperature), (*iii*) productivity (e.g., total phosphorus, conductivity, littoral area), (*iv*) natural reproduction and access (e.g., number of inlets and outlets), (*v*) anthropogenic control (e.g., stocking, distance to nearest road, road type), and (*vi*) biological (e.g., presence of competitors, presence of predators). In principle, all of these variables could be included in the set of control variables $\mathbf{X} = \{X_1, X_2, ..., X_n\}$. However, the more elements in **X**, the less likely it is that a given P lake will have an NP analog. Hence, if **X** includes too many variables, the number of P–NP pairs will be too small to yield any meaningful results. On the other hand, the more variables included, the better the control. So in selecting **X**, we must balance the benefit of increased control stringency against the cost of reduced sample size. In selecting control variables, we used the following criteria.

(*i*) The final set of selected variables **X** must be such that even at small D^2_{max}, i.e., $D^2_{max} \approx 0.5$, there were at least 10 P–NP pairs. This criterion is based solely on statistical power considerations. For n = 10 pairs with $\alpha = 0.05$, the power $(1 - \beta)$ associated with a large (≥0.80) effect size d under the one-tailed null hypothesis that average richness in P lakes is greater than or equal to that in NP lakes is only 0.53 (Faul and Erdfelder 1992). This effect size is very similar to that associated with the difference in average minnow richness between lakes with at least one piscivore and those without (absolute difference in mean richness = 0.85, $\sigma_{pooled} = 1.02$, d = 0.83; see results below). Thus, if the number of pairs is less than 10 at small D^2_{max}, our ability to detect even a large effect of piscivores is low.

(*ii*) All selected variables show some (functional or nonfunctional) bivariate relationship with minnow species richness or the presence of at least one of the 14 species considered here. This evidence could come from our own analyses, other ALSC work (e.g., Gallagher and Baker 1990; Christensen et al. 1990; Baker et al. 1993), or other studies (e.g., Chapleau et al. 1997; Whittier et al. 1997).

(*iii*) Where several potential control variables (e.g., maximum and mean depth, pH, acid-neutralizing capacity, surface conductance) showed strong (either linear or nonlinear) relationships, only one was selected, with less derived variables (variables that are measured directly or estimated directly from measured variables) selected over more derived variables (variables involving combinations of directly measured or estimated variables).

(*iv*) Variables whose (marginal) distributions differed significantly between lakes with and without piscivores were favored over those showing no differences. Because the sample size for each group is large, relatively small effect sizes are required to achieve statistical significance. Consequently, from those variables showing significant bivariate associations, we selected those having large effect sizes over those with small effect sizes.

Finally, watershed and zoogeographic variables specific to the Adirondack region were not considered here due to sample size constraints. The final set of selected control variables is shown in Table 1.

Table 1. Set of control variables **X** used in the study.

Variable	Piscivores		p
	Present	Absent	
Dissolved oxygen ($mg \cdot L^{-1}$)	6.90±0.157	6.99±0.098	0.63
pH	6.22±0.04	5.78±0.04	<0.001
Total phosphorus ($mg \cdot L^{-1}$)	0.017±0.001	0.021±0.001	0.003
Elevation (m)	427.0±7.6	491.1±4.9	<0.001
Surface area (ha)	22.2±2.4	9.0±0.76	<0.001
Littoral area (ha)	13.2±1.8	6.1±0.44	<0.001
Mean depth (m)	2.66±0.15	1.85±0.09	<0.001
Shoreline development	2.69±0.23	1.50±0.07	<0.001
Flush rate (no. of times·year^{-1})	38.3±7.49	56.8±5.92	0.001
No. of inlets	0.76±0.11	0.80±0.05	0.027
No. of outlets	0.43±0.05	0.67±0.04	<0.001

Note: Cell entries are means ± 1 SE for lakes with and without top piscivores and the associated two-tailed probabilities based on univariate Mann–Whitney comparisons.

Results

Piscivore distributions and minnow species richness

Most lakes with piscivores had only a single piscivore species present (Fig. 2). Overall, northern pike (58/131 = 44%) and largemouth bass (55/131 = 42%) were the most common species. In lakes with two piscivores present, the most common combination was two of northern pike, largemouth bass, and smallmouth bass (24/32 = 75%) (Fig. 3). In total, 114/131 = 87% of lakes had one of these three species.

Average minnow richness varied with the number of piscivore species present ($F_{3,502} = 21.6$, $p < 0.001$). As a group, lakes without top piscivores had an average minnow richness ($\bar{S} = 1.19 \pm 0.059$ (1 SE)) three times larger than lakes having one or more piscivore species present ($\bar{S} = 0.344 \pm 0.062$) (Fig. 2). Relative to lakes without piscivores, average

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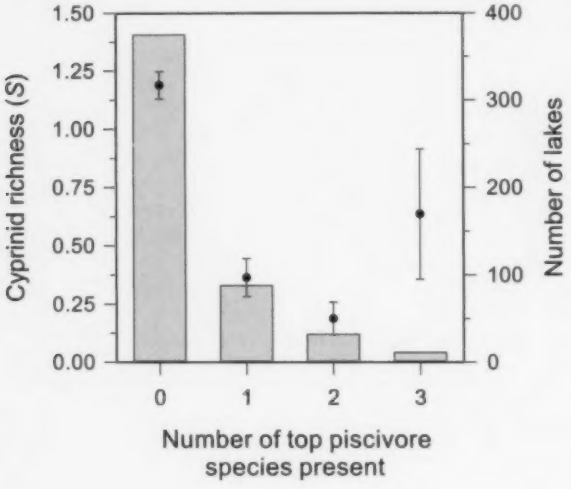
Fig. 2. Relationship between average minnow richness (±1 SD) of Adirondack lakes and the number of introduced piscivore species present (points and error bars). Also shown is the number of lakes in the sample having zero to three piscivore species (bars).



richness was significantly reduced in lakes with one or two piscivores (pairwise mean difference $d = -0.83$, $p < 0.001$ (Dunnet's test) and –1.00, $p < 0.001$, respectively) but not in lakes with three top piscivores (pairwise mean difference = –0.53, $p = 0.16$). The latter result is undoubtedly due to the relatively low power of this comparison as a consequence of the small number ($n = 11$) of lakes with three top piscivores (for $n_1 = 375$, $n_2 = 11$, mean difference between lakes = –0.53, and within-group (pooled) $\sigma = 0.86$, $d = -0.62$ and $1 - \beta = 0.65$ for a one-tailed t at $\alpha = 0.05$ (Faul and Erdfelder 1992)).

Minnow richness in Adirondack lakes was correlated with other physiochemical variables (e.g., Fig. 1), almost all of which covary with the presence of top piscivores (Table 1). Nonetheless, for any value of D^2_{max}, the distribution of the observed average P–NP difference Δ in minnow richness lay well to the left of the distribution of pairwise differences expected under the null hypothesis of no effect of piscivores on minnow richness (Fig. 4). As expected, the number of P–NP pairs increased with D^2_{max}, whereas the average difference declined asymptotically to about –0.85 (Fig. 5), the observed marginal pairwise difference in average richness. Average p ($\bar{p}$) values also decreased with D^2_{max} as a consequence of the increase in sample size, despite the countervailing (but weaker) influence of a decrease in effect size. But even at the smallest D^2_{max} (≈0.5), corresponding to very stringent controls but small sample size ($n \approx 10$), the type I probability associated with the one-tailed null exceeded 0.05 in at most 6/1000 randomization trials for any selection of P–NP pairs. Thus, while minnow richness is undoubtedly correlated with the other physiochemical and biotic factors used to define P–NP pairs, these correlations do not account for the reduced minnow richness (Fig. 2) of lakes with piscivores.

Minnow community composition

Minnows such as the creek chub and northern redbelly dace rarely occur in Adirondack lakes with piscivores, whereas the occurrence of other species such as the common shiner appears independent of the presence of piscivores (Fig. 6). These marginal effects are generally still present when other factors are taken into account. For species such as creek chub, the distribution of average differences lay well to the left of the distribution obtained under the null hypothesis at small D^2_{max}, whereas for the bluntnose minnow, the observed and null distributions were nearly identical. Thus, the former showed large (negative) average pairwise differences in presence scores at all values of D^2_{max}, whereas the latter showed insignificant differences (Fig. 7). Interestingly, the common shiner, whose marginal distribution was independent of the presence of piscivores (Fig. 6), showed significantly reduced occurrence in lakes with piscivores when other variables were controlled (Fig. 7).

Based on these analyses, we can classify the sensitivity of each species to the presence of piscivores (Table 2). First, there are those species that consistently exhibited large (negative) pairwise differences in scores at small D^2_{max}, such that the p values associated with the null hypothesis of no effect of piscivore presence were consistently <0.05 (class I). Indeed, for all species in this category except the common shiner, significant and large negative differences occurred over the full range of D^2_{max}. At the other extreme (class III) are those species such as bluntnose minnow that showed consistently negligible pairwise differences and high p values and, moreover, were sufficiently common in our sample to yield comparisons of at least moderate power ($1 - \beta > 0.50$) to detect the observed effect sizes. The third group (class II) comprises species for which there was some evidence of a negative effect of top piscivores, but the evidence was not strong. These are species that showed statistically significant ($p < 0.05$) negative pairwise differences in scores at larger values of D^2_{max} but only marginally significant ($0.05 < p < 0.15$) values at small D^2_{max} and that were present in at least 5% of the lakes in the sample. Finally, there are those species (class IV) that were so rare in the sample that any inference is unwarranted.

Discussion

Native minnow richness is dramatically reduced in Adirondack lakes with top piscivores, the latter comprising predominantly introduced species (northern pike, largemouth bass, and smallmouth bass). There is strong evidence that creek chub, northern redbelly dace, blacknose dace, and common shiner are less likely to occur in lakes with introduced piscivores; for pearl dace, the evidence is suggestive but not as strong. Of the 13 minnow species occurring in our sample, only bluntnose minnow and golden shiner appear to be unaffected by the presence of piscivores. The remaining six species were too rare in our sample to permit any meaningful inferences. We conclude that the observed differences in minnow species richness and community composition are due either to (*i*) direct or indirect effects of introduced piscivores per se leading to local extirpation of native minnows or to (*ii*) the effects of some other causal factor(s) whose distribution(s) is (are) strongly correlated with the presence of piscivores but not strongly correlated with any of the control variables we used.

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Fig. 3. Composition of top piscivore assemblages in lakes with (*a*) one, (*b*) two, or (*c*) three piscivore species. NP, northern pike; LMB, largemouth bass; SMB, smallmouth bass; CP, chain pickerel; W, walleye.

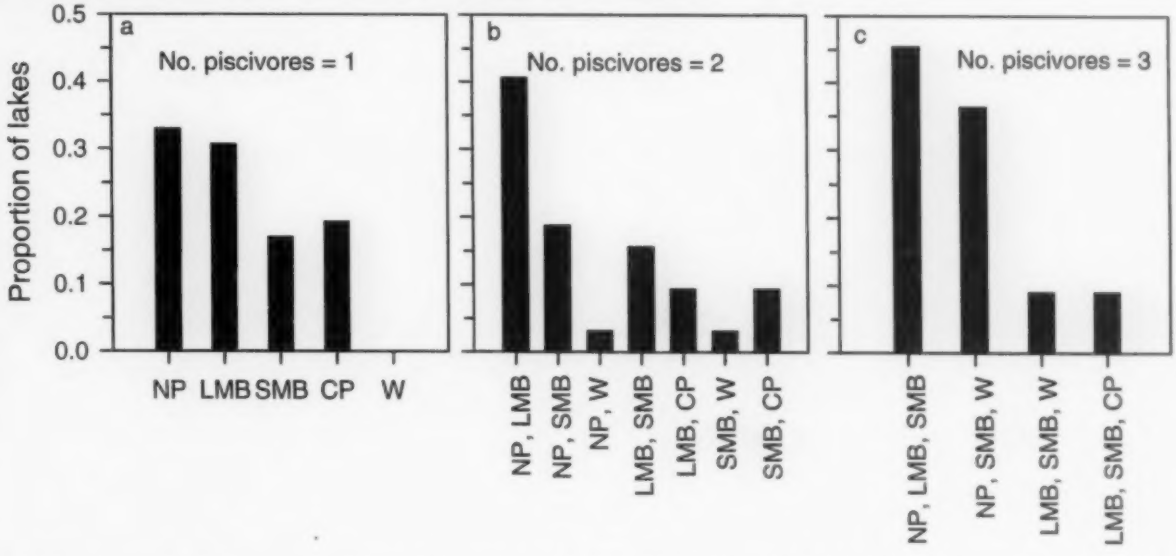
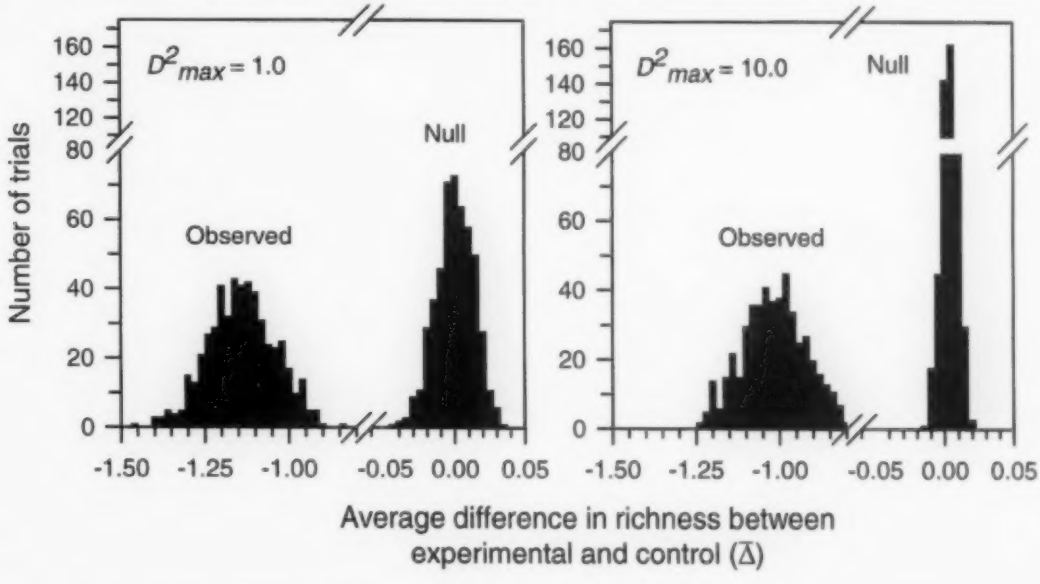


Fig. 4. Distribution of observed average pairwise differences $\bar{\Delta} = \Sigma_i(S_{i,P}) - S_{i,NP})/N(D^2_{max})$ in minnow richness between P and NP lakes and the distribution of differences expected under the null hypothesis of no difference. For each selection of P–NP pairs, the average pairwise difference in richness was calculated from 1000 randomizations, and the figure shows the distribution of these average differences over 1000 selections (trials) of P–NP sets of lakes. See the Appendix for a detailed description.



The latter is always a possibility. Baker and Gallagher (1990) noted that some potentially important factors influencing fish distributions are represented in the ALSC database only by surrogate (and perhaps inadequate) indices. Such factors include: minimum levels of dissolved oxygen and the potential for periodic dissolved oxygen deficits, maximum summer temperatures (and hence, habitat suitability for cold-water species), and, for some species, the availability of suitable spawning substrate. If these factors affect minnow community composition, are correlated with the presence of piscivores, but are not (or only weakly) correlated with any of our control variables, then the inference of a causal effect of introduced piscivores would be erroneous. Moreover, in the ALSC study, more than 50 physiochemical and biotic variables were measured, of which only 11 were used here. While an exhaustive analysis using all possible

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Fig. 5. Relationship between D^2_{max} and the average observed pairwise difference in minnow richness between lakes with and without piscivores ($\bar{\Delta}$) and the average number of independent pairs in the sample ($\bar{N}_T$), based on 1000 random selections of P–NP pairs. Standard errors for $\bar{\Delta}$ were less than 0.05 for all values of D^2_{max}. See the Appendix for further explanation.

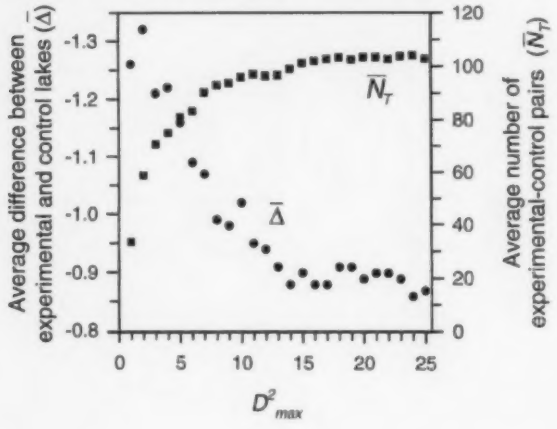
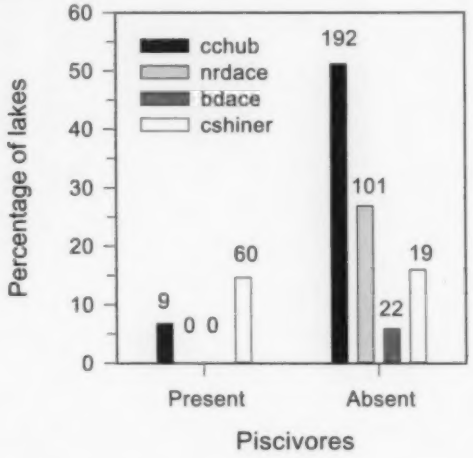


Fig. 6. Distribution of creek chub (cchub), northern redbelly dace (nrdace), blacknose dace (bdace), and common shiner (cshiner) in lakes with and without top piscivores. The number of lakes in each category for which the species was present is also given. The first three species occur significantly more frequently in lakes without piscivores (Yates corrected χ^2 = 78.8, 44.4 ($p < 0.001$), and 6.69 ($p = 0.01$), respectively), whereas the marginal occurrence of common shiner is independent of the presence of piscivores ($\chi^2 = 0.07$, $p = 0.79$).



subsets of the 50 variables is not feasible, we ran our analyses using several dozen different (not mutually exclusive) control variable sets, and in all cases, the conclusions were similar to those reported here.

Loss of species may be a gradual process, and in the Adirondacks, fish communities have been exposed to a wide range of anthropogenic stressors for decades. Our analytical approach controls for the effects of factors other than piscivores by using values measured at the time the ALSC data were collected in the mid-1980s. However, these values may be quite different from what they were in the past (Magnuson et al. 1994, 1998). For example, in the Adirondacks, there is good paleolimnological evidence that some lakes with quite similar pHs in the 1980s were quite different in the early to mid-1900s; some lakes have become acidic over the last few decades, whereas others are naturally acidic (Cumming et al. 1994).

This may lead to problems in inference. For example, minnows are more sensitive to acid stress than bass and pike (Rahel and Magnuson 1983). Hence, we expect lakes that have had a long history of acidification to have reduced current minnow richness compared with those that have only recently begun to acidify, even if at the time of the ALSC survey, their pHs were very similar. If the former were also more likely to have piscivores at the time of the ALSC survey (because piscivores are more resistant to acid stress), we would expect to see reduced minnow richness in lakes with piscivores compared to those without, as observed. But the conclusion that this effect is due to piscivores would be incorrect: the causal factor underlying the observed pattern is acidification, and the erroneous inference arises because while we have controlled for current acid stress levels (as indexed by pH), we have not controlled for differences in acidification histories.

These caveats notwithstanding, the simplest explanation for our results is that the introduction of top piscivores results in local extirpation of native minnows. It is, in our view, significant that of the 13 minnow speciess occurring in our sample, only golden shiner and bluntnose minnow appear to be unaffected by the presence of top piscivores. Neither of these species is native to the Adirondacks, but both are common baitfish that have been widely introduced outside their native range (Whittier et al. 1997). For two other small-bodied fish species (central mudminnow (*Umbra limi*) and rainbow smelt (*Osmerus mordax*) present in a sufficient number of lakes to allow meaningful inferences, our approach provides strong evidence that neither is affected by the presence of piscivores (Table 2). Rainbow smelt is certainly nonnative, and although the central mudminnow is considered native to the Champlain watershed, it has been extensively introduced throughout the rest of the Adirondack region (NYSDEC 1927–1939). Thus, all native small-bodied species for which there is a sufficient sample to permit meaningful inferences show evidence of a negative impact of top piscivores, whereas for all small, soft-finned nonnative species, there is strong evidence that they are unaffected by piscivores. This leads us to speculate that local populations of nonnative species are less likely to be extirpated by piscivores than are native species simply because, for the former, immigration or colonization events are much more frequent, especially if they are common baitfish (as are golden shiner and bluntnose minnow) or are less sensitive to other stresses such as acidification (e.g., golden shiner) (Gallagher and Baker 1990; Baker et al. 1993; Whittier and Kincaid 1999). It is also possible that because the nonnative species have long histories of occurrence (in other regions) with the dominant top piscivores in the Adirondacks, their coevolution may have rendered them relatively insensitive to predation.

Our results add to the growing body of evidence that

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Fig. 7. (*a*) Average difference $\bar{\Delta}$ in presence–absence scores between lakes with and without piscivores for northern redbelly dace (circles), common shiner (triangles), and bluntnose minnow (squares) in relation to D^2_{max}, based on 1000 random selections of P–NP pairs and (*b*) one-tailed average type I probabilities $\bar{p}$ associated with the null hypothesis that the probability of occurrence is as great or greater in piscivore lakes compared with those without piscivores.

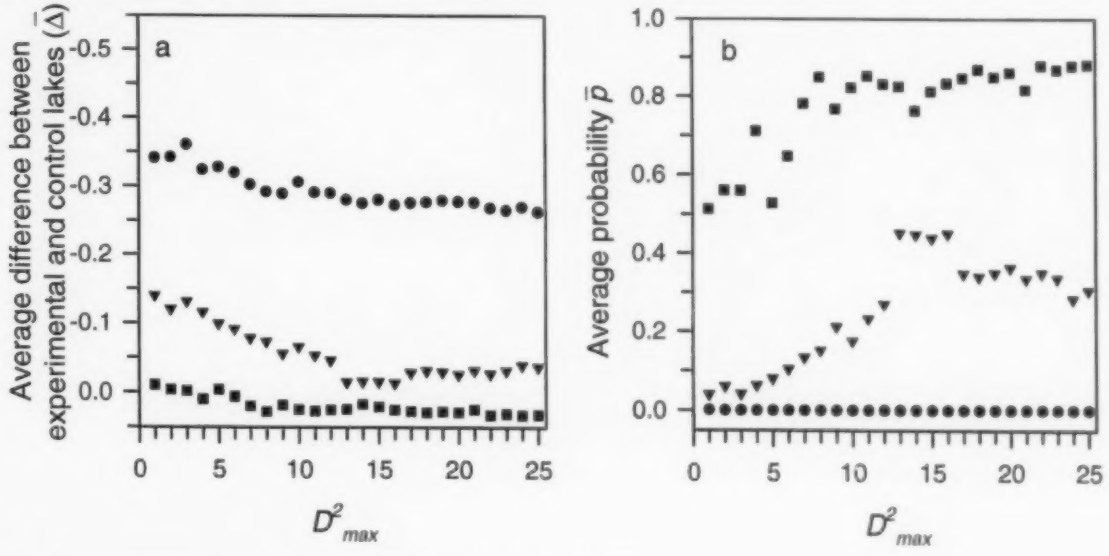

Table 2. Sensitivity of small-bodied species to the presence of top piscivores.

Class I	Class II	Class III	Class IV
Native cyprinids	Native cyprinids	Nonnative cyprinids	Native cyprinids
Creek chub (*Semotilus atromaculatus*)	Pearl dace (*Margariscus margarita*)	Golden shiner (*Notemigonus crysoleucas*)	Lake chub (*Couesius plumbeus*)
Northern redbelly dace (*Phoxinus eos*)		Bluntnose minnow (*Pimephales notatus*)	Fathead minnow (*Pimephales promelas*)
Blacknose dace (*Rhinichthys atratulus*)			Finescale dace (*Phoxinus neogaeus*)
Common shiner (*Luxilus cornutus*)		Nonnative small-bodied species	Longnose dace (*Rhinichthys cataractae*)
		Central mudminnow(*Umbra limi*)	Cutlips minnow (*Exoglossum maxillingua*)
		Rainbow smelt (*Osmerus mordax*)	Bridle shiner (*Notropis bifrenatus*)
			Nonnative small-bodied species
			Banded killifish (*Fundulus diaphanus*)

Note: Class I are those species for which there is strong evidence of sensitivity, class II are those for which there is some evidence of sensitivity, class III are those for which there is strong evidence of no (or little) sensitivity, and class IV are those too rare in the sample to permit any meaningful inference.

piscivore introductions result in reduced local and regional minnow biodiversity. Tonn and Magnuson (1982) showed that minnows and mudminnows were common in northern Wisconsin lakes where winterkill eliminated northern pike and centrarchids but were largely absent from lakes with piscivores. Robinson and Tonn (1989) concluded that in small Alberta lakes, piscivory is a major factor structuring fish assemblages, with lakes with piscivores showing reduced minnow diversity. Chapleau et al. (1997) also showed reduced minnow richness in small lakes in Gatineau Park, Quebec, compared with lakes with introduced piscivores (primarily smallmouth bass and northern pike). In the 1930s, minnow diversity was considerably greater in Adirondack lakes to which bass (*Micropterus* spp.) had not yet been introduced compared with lakes where they had been introduced (NYSDEC 1927–1939). Moreover, lakes for which there is good evidence of minnow extirpations since the 1930s were more likely to have had introduced piscivores present at the time of the ALSC surveys in the mid-1980s (Baker et al. 1993). Recently, Whittier et al. (1997) presented data suggesting that a major factor involved in regional-scale minnow biodiversity losses in the northeastern United States is the introduction of top piscivores.

This correlational evidence is complemented by some di-

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rect experimental work. He and Kitchell (1990) documented reductions in minnow biomass in a small lake following introduction of northern pike. This reduction was apparently a result of both the direct effects of predation and indirect effects including habitat shifts in an attempt to avoid predators, increased emigration rates, and a decrease in the mean size of species considered to be most vulnerable to predation. In another manipulation study, He and Wright (1992) provided evidence that increased emigration by minnows under predation pressure might be density dependent.

Most of the studies cited above involved small lakes ranging from less than 1 to a few dozen hectares in size. In the present study, lake area varied from 0.1 to 160 ha (average 12.4 ± 19.8 (1 SD)), with fully 95% being less than 50 ha. Chapleau et al. (1997) showed reduced minnow richness in small lakes (<100 ha) with introduced piscivores compared with those without but could not detect any differences in richness among larger lakes. These results, combined with evidence that habitat heterogeneity and extent are an important determinant of fish assemblages (e.g., Eadie and Keast 1984; Jackson and Harvey 1989; Pierce et al. 1994), suggest that minnows are more vulnerable to predation in small lakes because habitat heterogeneity is low and refuges from predation are few and far between (Tonn and Paszkowski 1986; Tonn et al. 1992).

In our view, the weight of evidence from both correlational studies such as the present one and experimental studies (Harvey et al. 1988; Harvey 1991; He and Wright 1992) strongly suggests that in small (i.e., <50–100 ha) temperate lakes, introduction of top piscivores puts native minnow populations at high extinction risk. If (as we do) one believes that "aquatic ecosystems should be managed to ensure long-term sustainability of native fish stocks" (Olver et al. 1995, p. 1588), and furthermore, if one subscribes (which we also do) to the precautionary principle, then this weight of evidence leads to three important management implications for small temperate lakes with extant native minnow assemblages: (*i*) fish and wildlife agencies should not engage in piscivore stocking in such lakes regardless of pressure from sportfishing or other lobbies, (*ii*) such agencies should not permit (where they have the jurisdictional authority to do so) or actively discourage (where jurisdictional authority is lacking) piscivore stocking by nongovernmental organizations such as hunting and angling clubs, and (*iii*) such agencies should make concerted efforts to communicate to the public the risks to native minnow communities posed by the accidental or deliberate introduction of top piscivores and provide practical suggestions as to how these risks can be reduced.

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References

Angermeier, P.J. 1995. Ecological attributes of extinction-prone species: loss of freshwater fishes of Virginia. Conserv. Biol. **9**: 143–158.

Baker, J., and Gallagher, J. 1990. Fish communities in Adirondack lakes. *In* Adirondack Lake Survey: an interpretive analysis of fish communities and water chemistry, 1984–1987. *Edited by* J.P. Baker and S.A. Gherini. Adirondack Lake Survey Corporation, Ray Brook, N.Y. pp. 3-1 to 3-10.

Baker, J.P., Gherini, S.A., Christensen, S.W., Driscoll, C.T., Gallagher, J., Munson, R.K., Newton, R.M., Reckhow, K.H., and Schofield, C.L. 1990. Adirondack Lake Survey: an interpretive analysis of fish communities and water chemistry, 1984–1987. Adirondack Lake Survey Corporation, Ray Brook, N.Y.

Baker, J.P., Warren-Hicks, W.J., Gallagher, J., and Christensen, S.W. 1993. Fish population losses from Adirondack lakes: the role of surface water acidity and acidification. Water Resour. Res. **29**: 861–874.

Chapleau, F., Findlay, C.S., and Szenasy, E. 1997. Impact of piscivorous fish introductions on fish species richness of small lakes in Gatineau Park, Quebec. Ecoscience, **4**: 259–268.

Christensen, S., Beauchamp, J.L., Shaakir-Ali, J.A., Coes, J.M., Baker, J.P., Smith, E.P., and Gallagher, J. 1990. Patterns of fish distribution in relation to lake/watershed characteristics: regression analyses and diagnostics. *In* Adirondack Lake Survey: an interpretive analysis of fish communities and water chemistry, 1984–1987. *Edited by* J.P. Baker and S.A. Gherini. Adirondack Lake Survey Corporation, Ray Brook, N.Y. pp. A-i to A-62.

Cumming, B.F., Davey, K.A., Smol, J.P., and Birks, H.J.B. 1994. When did acid-sensitive Adirondack lakes (New York, U.S.A.) begin to acidify and are they still acidifying? Can. J. Fish. Aquat. Sci. **51**: 1550–1568.

Draper, N., and Smith, H. 1981. Applied regression analysis. John Wiley & Sons, New York.

Eadie, J.McA., and Keast, A. 1984. Resource heterogeneity and fish species diversity in lakes. Can. J. Zool. **62**: 1689–1695.

Faul, F., and Erdfelder, E. 1992. GPOWER: a priori, post-hoc and compromise power analyses for MS-DOS (computer program). Department of Psychology, Bonn University, Bonn, Germany.

Gallagher, J., and Baker, J. 1990. Current status of fish communities in Adirondack lakes. *In* Adirondack Lake Survey: an interpretive analysis of fish communities and water chemistry, 1984–1987. *Edited by* J.P. Baker and S.A. Gherini. Adirondack Lake Survey Corporation, Ray Brook, N.Y. pp. 3-11 to 3-44.

Haines, T.A., and Baker, J.P. 1986. Evidence of fish population responses to acidification in the eastern United States. Water Air Soil Pollut. **31**: 605–629.

Harig, A.L., and Bain, M.B. 1998. Defining and restoring biological integrity in wilderness lakes. Ecol. Appl. **8**: 71–87.

Harvey, B.C. 1991. Interactions among stream fishes: predator-induced habitat shift and larval survival. Oecologia, **87**: 29–36.

Harvey, B.C., Cashner, R.C., and Matthews, W.J. 1988. Differential effects of largemouth and smallmouth bass on habitat use by stoneroller minnows in stream pools. J. Fish Biol. **33**: 481–487.

He, X., and Kitchell, J.F. 1990. Direct and indirect effects of predation on a fish community: a whole-lake experiment. Trans. Am. Fish. Soc. **119**: 825–835.

He, X., and Wright, R.A. 1992. An experimental study of piscivore–planktivore interactions: population and community responses to predation. Can. J. Fish. Aquat. Sci. **49**: 1176–1183.

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Hughes, R.M., and Noss, R.F. 1992. Biological diversity and biological integrity: current concerns for lakes and streams. Fisheries (Bethesda), **17**: 11–19.

Jackson, D.A., and Harvey, H.H. 1989. Biogeographic associations in fish assemblages: local vs. regional processes. Ecology, **70**: 1472–1484.

Magnuson, J.J., Benson, B.J., and McLain, A.S. 1994. Insights on species richness and turnover from long-term ecological research: fishes in north temperate lakes. Am. Zool. **34**: 437–451.

Magnuson, J.J., Tonn, W.M., Banerjee, A., Toivonen, J., Sanchez, O., and Rask, M. 1998. Isolation vs. extinction in the assembly of fishes in small northern lakes. Ecology, **79**: 2941–2956.

Matuszek, J.E., and Beggs, G.L. 1988. Fish species richness in relation to lake area, pH, and other abiotic factors in Ontario lakes. Can. J. Fish. Aquat. Sci. **45**: 1931–1941.

Matuszek, J.E., Goodier, J., and Wales, D.L. 1990. The occurrence of Cyprinidae and other small fish species in relation to pH in Ontario lakes. Trans. Am. Fish. Soc. **119**: 850–861.

New York State Department of Environmental Conservation (NYSDEC). 1927–1939. Biological surveys (12 volumes). New York State Department of Environmental Conservation, Albany, N.Y.

Olver, C.H., Shuter, B.J., and Minns, C.K. 1995. Towards a definition of conservation principles for fisheries management. Can. J. Fish. Aquat. Sci. **52**: 1584–1594.

Persson, L. 1997. Competition, predation and environmental factors as structuring forces in freshwater fish communities: Sumari (1971) revisited. Can. J. Fish. Aquat. Sci. **54**: 85–88.

Pierce, C.L., Rasmussen, J.B., and Leggett, W.C. 1994. Littoral fish communities in southern Quebec lakes: relationships with limnological and prey resource variables. Can. J. Fish. Aquat. Sci. **51**: 1128–1138.

Rago, P.J., and Wiener, J.G. 1986. Does pH affect species richness when lake area is considered? Trans. Am. Fish. Soc. **115**: 438–447.

Rahel, F.J. 1986. Biogeographic influences on fish species composition of northern Wisconsin lakes with applications for lake acidification studies. Can. J. Fish. Aquat. Sci. **43**: 124–134.

Rahel, F.J., and Magnuson, J.J. 1983. Low pH and the absence of fish species in naturally acidic Wisconsin lakes: inference for cultural acidification. Can. J. Fish. Aquat. Sci. **40**: 3–9.

Robinson, C. L. K., and Tonn, W.M. 1989. Influence of environmental factors and piscivory in structuring fish assemblages of small Alberta lakes. Can. J. Fish. Aquat. Sci. **46**: 81–89.

Scharf, F.S., Juanes, F., and Sutherland, M. 1998. Inferring relationships from the edges of scatter diagrams: a comparison of regression techniques. Ecology, **79**: 448–460.

Scott, M.C., and Hall, L.W. 1997. Fish assemblages as indicators of environmental degradation in Maryland coastal plain streams. Trans. Am. Fish. Soc. **126**: 349–360.

Terrell, J.W., Cade, B.S., Carpenter, J., and Thompson, J.M. 1996. Modeling stream fish habitat limitations from wedge-shaped patterns of variation in standing stock. Trans. Am. Fish. Soc. **125**: 104–117.

Tonn, W.M., and Magnuson, J.J. 1982. Patterns in the species composition and richness of fish assemblages in northern Wisconsin lakes. Ecology, **63**: 1149–1166.

Tonn, W.M., and Paszkowski, C.A. 1986. Size-limited predation, winterkill, and the organization of *Umbra–Perca* fish assemblages. Can. J. Fish. Aquat. Sci. **43**: 194–202.

Tonn, W.M., Paszowski, C.A., and Holopainen, I.J. 1992. Piscivory and recruitment: mechanisms structuring prey populations in small lakes. Ecology, **73**: 951–958.

Whittier, T.R., and Kincaid, T.M. 1999. Introduced fish in northeast U.S.A. lakes: regional extent, dominance, and effect on native species richness. Trans. Am. Fish. Soc. **128**: 769–783.

Whittier, T.R., Halliwell, D.B., and Paulsen, S.G. 1997. Cyprinid distributions in northeast U.S.A. lakes: evidence of regional-scale minnow biodiversity losses. Can. J. Fish. Aquat. Sci. **54**: 1593–1607.

Appendix. Analytical procedure

(1) Determine the set of lakes in the sample with at least one piscivore species present, i.e., piscivore (P) lakes. This set has N_T elements.

(2) Define the set of lake characteristics (variables) $\mathbf{X} = X_1, X_2, ..., X_n$ used to determine how similar a given nonpiscivore (NP) lake is to a specified P lake. These variables are given in Table 1.

(3) Set the maximum Mahalanobis distance $D^2_{\max}$ based on the set of variables defined in step 2. Because this maximum distance is arbitrary, we consider it an adjustable parameter. As $D^2_{\max}$ decreases, control stringency increases (i.e., the more similar a NP lake must be to the specified P lake in order to be designated as a reference lake), but sample size decreases. An infinite $D^2_{\max}$ means that any NP lake could be designated as a reference for any P lake: statistically, this is equivalent to a paired sample comparison of the marginal average minnow richnesses of lakes with and without piscivores.

(4) Choose a random sequence of P lakes $L_1, L_2, ..., L_{N_T}$. Beginning with the first lake in the sequence, choose a single lake at random from the set of all NP lakes satisfying the $D^2_{\max}$ criterion for this lake. Check that this randomly selected lake has not already been selected as a reference for some other P lake chosen earlier in the sequence (this condition is required to ensure that all P–NP pairs are indeed unique, i.e., independent) and repeat for all P lakes in the sequence. The result is a set of $N(D^2_{\max}) \leq N_T$ P–NP pairs, since some P lakes will have no NP lakes satisfying both the $D^2_{\max}$ and independence criteria. Note that because we require that all P–NP pairs be unique, different sequences of P lakes will generally yield different sets of P–NP pairs and different sample sizes (number of pairs).

(5) Calculate the difference in minnow richness $\Delta_i = S_{i,\mathrm{P}} - S_{i,\mathrm{NP}}$ for all $N(D^2_{\max})$ pairs i of P and NP lakes obtained in step 4. Then calculate the average difference for the sample $\overline{\Delta}_{D^2_{\max}} = \Sigma_i \Delta_i / N(D^2_{\max})$.

(6) Randomize the set of P–NP lakes obtained in step 4 by randomly assigning each member of the P–NP pair to one or the other category. Calculate the difference in richness between randomized pairs and repeat for all $N(D^2_{\max})$ pairs. This yields a set of $N(D^2_{\max})$ differences $\{\Delta^0_i\}$. Then calculate the average difference $\overline{\Delta}^0_{D^2_{\max}} = \Sigma_i \Delta^0_i / N(D^2_{\max})$ for the jth randomization trial.

(7) Repeat step 6 for $j = 1,..., 1000$ randomization trials, each randomization trial representing a selection of $N(D^2_{\max})$ pairs of lakes obtained in step 4 and with members of the pair being randomly assigned to either the P or the NP category. This results in a set of 1000 average differences $\{\overline{\Delta}^0_{j,D^2_{\max}}\}$, one for each randomization. Generate the cumulative distribution $F(\overline{\Delta}^0_{D^2_{\max}})$ and calculate

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the proportion of trials yielding an average difference between P and NP pairs less than or equal to the observed average difference $\overline{\Delta}_{D^2_{max}}$ calculated in step 5. This proportion is the probability p of obtaining the observed average difference between P and NP lakes under the (one-tailed) null hypothesis that the average richness of the former is greater than or equal to that of the latter. We specify a one-tailed null because the biological hypothesis is that the introduction of top piscivores leads to reduced minnow diversity.

(8) Repeat steps 4–7 for k = 1,..., 1000 Monte Carlo trials, each trial corresponding to a random sequence of P lakes and a random selection of corresponding NP lakes, subject to the criteria outlined in step 3. This random selection of NP lakes is necessary because for larger Mahalanobis distances, a given P lake may have several possible NP analogs. Each trial k = 1,..., 1000 generates a probability p, defined in step 7 above, which we denote p_k. The set of 1000 trials therefore generates a distribution of p values $\phi(p)$, from which we can calculate various moments, e.g., the mean $\overline{p}$.

(9) Repeat steps 3–8 for different D^2_{max}, with small D^2_{max} indicating relatively close matching of lake characteristics (i.e., high control stringency).

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Influence of moon phase on acoustic estimates of the abundance of fish performing daily horizontal migration in a small oligotrophic lake

Nathalie Gaudreau and Daniel Boisclair

Abstract: We tested the influence of the moon phase on the relative number of fish performing daily horizontal migrations and we used this information to develop a sampling strategy that accounted for expected effects of the moon phase on fish behavior. Hydroacoustic surveys were performed over 3 days at each moon phase for the months of July and August (from the day before to the day after the new moon, the first and last quarter moon, and the full moon). Dace (*Phoxinus eos* × *Phoxinus neogaeus*) migrated from the littoral towards the pelagic zone at dusk and returned to the littoral zone at dawn. Their relative abundance (FRA) in the pelagic zone averaged 0.1 fish echo·m^{-3} during midday and 17.0 fish echoes·m^{-3} at night. The number of dace performing horizontal movements was eightfold larger during new-moon than full-moon nights. Simulations indicated that sampling fish populations six times during a 2-month period (once at full, new, and quarter moon; once during the day and once during the night) provided the most accurate estimates of FRA.

Résumé : Nous avons vérifié l'influence des phases lunaires sur le nombre de poissons qui effectuent des migrations journalières horizontales. Cette information a été utilisée pour développer des stratégies d'échantillonnage qui tiennent compte des effets potentiels des phases lunaires sur le comportement des poissons. Des relevés hydroacoustiques ont été effectués sur une période de 3 jours à chaque phase lunaire des mois de juillet et août (du jour précédent au jour suivant la nouvelle, la pleine et les premier et dernier quart de lune). Les ménés (*Phoxinus eos* × *Phoxinus neogaeus*) migraient de la zone littorale vers la zone pélagique au crépuscule et retournaient vers la zone littorale à l'aube. Leur abondance relative (FRA) dans la zone pélagique a été en moyenne de 0.1 écho de poisson·m^{-3} en plein jour et de 17.0 écho de poisson·m^{-3} la nuit. Le nombre de ménés qui ont migré horizontalement a été huit fois plus élevé durant les nuits de nouvelle lune comparativement aux nuits de pleine lune. Des simulations ont démontré qu'échantillonner les populations de poissons six fois sur une période de 2 mois (une fois à la pleine, à la nouvelle et au quart de lune; une fois durant le jour et une fois durant la nuit) fournit les estimations de FRA les plus exactes.

Introduction

Abundance is an important economical and ecological characteristic of fish populations. Abundance largely sets the limits of the number of fish that might be removed from exploited populations (Hilborn and Walters 1992). Abundance also affects the magnitude of direct and indirect interactions between fish and other components of aquatic food webs. For instance, fish abundance defines the intensity of the foraging pressure applied by fish and their effect on prey abundance, size distribution, taxonomic composition, and aggregation pattern (Mills et al. 1987; Johannes 1993; Jeppesen et al. 1997). Similarly, fish abundance and distribution have been hypothesized to affect their role as nutrient recycling agents. Through a combination of migration and excretory activities, fish have been argued to transport phosphorus and nitrogen between specific areas of lakes and hence to qualitatively and quantitatively affect primary producers (Baumann and Kitchell 1974; Schindler et al. 1996; Vanni 1996). Accurate information on fish abundance, distribution, and migration therefore represents a prerequisite to adopt sound management decisions and to test hypotheses concerning the role of fish in aquatic systems.

Spatial and temporal heterogeneity represents the most challenging aspect of the study of fish abundance. The recent advent of relatively rapid and nonintrusive hydroacoustic techniques has allowed researchers to survey fish abundance at spatial and temporal scales appropriate for studying biological processes (MacLennan and Simmonds 1992; Rose 1992; Misund 1997). These techniques have been used to study variations in fish abundance during daily horizontal (Bohl 1980; Gliwicz and Jachner 1992; Gauthier and Boisclair 1997) and vertical migrations (Begg 1976; Levy 1987; Eckmann and Imbrock 1996). Seasonal migrations have also been described (Imbrock et al. 1996; Lucas et al. 1998). Such recurrent and predictable patterns represent key structural elements that facilitate the development of appropriate sampling designs for estimating fish abundance and its spatial and temporal heterogeneity. There are indications, however, that in addition to day and season, other temporal scales may be relevant for studying fish abundance and distribution patterns.

Luecke and Wurtsbaugh (1993) showed that the moon

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phase affects the daily vertical migration of fish. They demonstrated that fish biomass and density acoustically sampled in the pelagic zone of Bear Lake at night were 45 and 51% lower, respectively, during the full moon than during the new moon. On full-moon nights, fish presumably stayed closer to the bottom and were less susceptible to acoustic detection. Similarly, while acoustically studying daily horizontal migration of fish in Lake Croche, Comeau and Boisclair (1998) observed three times fewer fish than average on nights close to the full moon. However, their study covered a period of only 15 consecutive days and consequently did not include a complete set of moon phases. Both studies indicate, however, that the specific moon phase selected for sampling could significantly affect estimates of fish abundance and our perspective on the role of fish in lakes.

The objectives of our work were to (*i*) test the effect of the moon phase on acoustic estimates of the relative numbers of fish performing daily horizontal migrations and (*ii*) develop a sampling strategy that would account for expected effects of the moon phase on fish behavior and abundance.

Material and methods

Study lake

Hydroacoustic surveys were performed in a bay of the eastern basin of Lake Croche (bay characteristics: surface area = 0.38 ha, maximum depth = 7 m). Lake Croche is a small and shallow oligotrophic lake located at the Station de Biologie des Laurentides de l'Université de Montréal (Fig. 1). In May, the stratification of the water temperature sets up gradually. Surface temperatures reach a maximum of 24°C in mid-August, while bottom temperatures fluctuate between 6 and 8°C. From early July to late August, anoxic conditions prevail below a depth of approximately 4–6 m. The fish community of the littoral zone is numerically dominated by a hybrid between northern redbelly dace and finescale dace (*Phoxinus eos* × *Phoxinus neogaeus*) and by pumpkinseed (*Lepomis gibbosus*). Creek chub (*Semotilus atromaculatus*) and white sucker (*Catostomus commersoni*) are also found in the littoral zone. Piscivores such as brook trout (*Salvelinus fontinalis*) and lake trout (*Salvelinus namaycush*) occupy the pelagic zone. Lake Croche was selected for study because previous work indicated that hybrid dace perform daily horizontal migrations from the littoral to the pelagic zone at dusk and from the pelagic to the littoral zone at dawn (Gauthier and Boisclair 1997; Comeau and Boisclair 1998). This migration increased the number of fish present in the pelagic zone at night, compared with midday, by a factor of 9 during full moon and a factor of 24 during last quarter moon. No vertical migrations were observed, presumably because of the anoxic conditions below the epilimnion.

Field procedure

Fish distribution patterns were investigated with a single-beam hydroacoustic system (SIMRAD EY-200P, frequency = 200 kHz). Signals were recorded using a digital audiotape (TEAC model DAP 20). The acoustic system was calibrated with a standard copper sphere (target strength (TS) = −44.1 dB). During calibration, acoustic parameters were set to the values implemented for our fish surveys (pulse duration = 0.3 ms; ping rate = $2 \cdot s^{-1}$; receiver gain = 3; $40 \log R$ TVG function starting at 2.0 m). The theoretical beam angle provided by SIMRAD for our transducer is 3.5° (–3 dB half-beam angle). The effective beam angle was assessed in the field for two kinds of targets: the calibration sphere and swimming hybrid dace (Gauthier et al. 1997). Dace were selected for this procedure because they represent the only fish species performing daily migrations in Lake Croche. The size of dace used during this field assessment (6 cm total length (TL)) corresponded to the average length of the migrating dace (range = 4.5–6.5 cm; Gauthier et al. 1997). TS for dace with a similar length (5.0 cm TL) ranged from –52 to –42 dB (Comeau and Boisclair 1998). Targets were maintained at 0.8 m below the surface using a wire anchored to the bottom and attached to a buoy at the surface. Targets were positioned at 10, 20, 30, and 40 m from the transducer. The transducer was installed at the extremity of a pole, lowered 0.8 m below the surface, and set such that the axis of the acoustic beam was parallel to the surface of the lake (horizontal scanning approach; Kubecka 1996; Gauthier et al. 1997). As we rotated the pole, the angular position of the target was noted when entering and exiting the acoustic beam using a protractor centered at the axis of the pole holding the transducer. The effective beam angle averaged 11° (±0.5°). That value remained constant for the two kinds of targets and for the range of distances between the target and the transducer.

Acoustic sampling was performed from a floating platform anchored 50 m from shore using the horizontal scanning approach (Fig. 1). The transducer was mounted at the extremity of a pole, lowered 0.8 m below the surface, and directed towards the lakeshore. The pole was installed on an axle that enabled us to rotate the transducer in the horizontal plane. A sampling session was performed every 4 h (09:00, 13:00, 17:00, 21:00, 01:00, 05:00) during 3 consecutive days of a given week for 7 successive weeks (from July 14 to August 30, 1996). Hence, our sampling schedule allowed us to survey fish abundance and distribution over almost two complete moon cycles. The 3 consecutive days of a sampling week were selected to span from the day before to the day after a specific moon phase (new moon, first quarter, full moon, and last quarter). A sampling session consisted of the scanning of four adjacent sectors of 11° originating from the pole supporting the transducer. Each adjacent sector was separated by 5° to prevent the possible overlap of sectors due to movement of the floating platform during moderately windy conditions (Fig. 1). Each sector was insonified during 3 min. This decision of insonifying sectors during 3 min was, to some extent, arbitrary. We had used 3-min sampling periods in previous studies and this had allowed us to obtain data adequate for analysis of distribution and migration patterns and from which we could extract intuitively clear and statistically significant patterns (Gauthier and Boisclair 1997; Gauthier et al. 1997; Comeau and Boisclair 1998). Moreover, since sectors were always insonified for 3 min in this study, if ever a bias was introduced, it was constant among sampling units and should not have prevented us from discriminating patterns at this particular temporal scale.

Thunderstorms and heavy rain occasionally prevented sampling because important precipitation could be acoustically detected (>0.9 $mm \cdot h^{-1}$). As a consequence, only 107 of the 126 sampling sessions planned could be effectively completed. We were interested in quantifying surface light intensity. A Licor™ lightmeter and a Biospherical quantummeter were used, but both instruments were not sensitive enough to detect light at night. A method for calculating moonlight illuminance at the earth's surface was then found, but it required data on type, altitude, and height of clouds, which we could not obtain (Austin et al. 1976). Finally, we decided to evaluate the percentage of sky covered by clouds. Hence, percent cloudiness and rain were noted at each sampling session. Luminosity during sampling sessions performed at 05:00 or 21:00 was not constant from one sampling week to another due to variation in timing of sunrise and sunset. Twilight conditions prevailed during sampling sessions conducted at 05:00 for the first 4 sampling weeks and at 21:00 for the first 2 weeks. Sampling at 05:00 and 21:00 took place in complete darkness for the last 3 (05:00) and last 5 sampling weeks (21:00). Care was taken to use minimum artificial lighting when surveying in total darkness.

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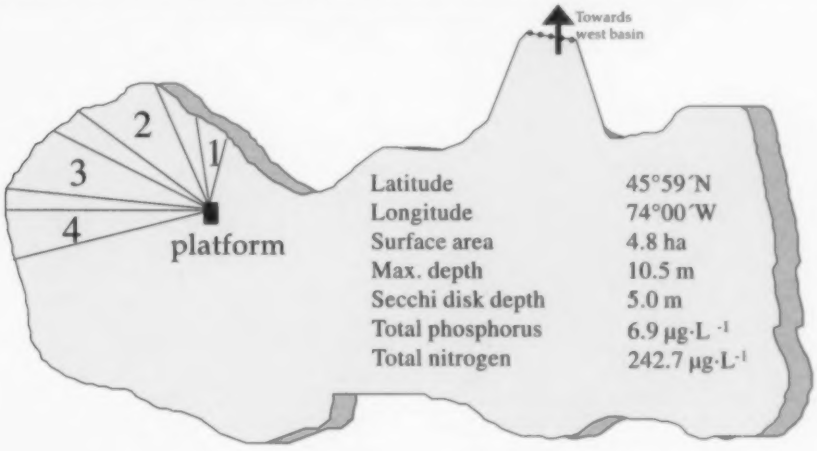
Fig. 1. Study site (west basin of Lake Croche not shown) with morphological and limnological characteristics. The platform used to perform hydroacoustic surveys as well as the four sectors that were insonified are illustrated.



Biological interpretation of acoustic signals

The hydroacoustic beam intersected the bottom of the lake at the 3- to 4-m isobath. This limit is further referred to as the hydroacoustic shore. Consequently, targets located nearshore in waters shallower than 3–4 m could not be discriminated from the background. Hence, targets recorded were considered as being inside the pelagic zone (or outside the littoral zone). The hydroacoustic shore was at approximately 15 m from the lakeshore and 35–50 m from the platform depending on the sector.

The frequency of our acoustic system (200 kHz) allowed echoes from zooplankton invertebrates such as *Holopedium* to be detected. Therefore, signals originating from zooplankton had to be eliminated from our analyses. Since no cutoff point was observed in the TS distribution of our recordings, we had to define one. Relationships between TS and body length have been developed using the vertical scanning approach but may not be applicable in horizontal scanning (MacLennan and Simmonds 1992; but see Kubecka 1994). However, during previous field assessment of TS using the horizontal scanning approach, a break in the TS distribution of *P. eos* × *P. neogaeus* 5.0 cm TL had been observed at –52 dB (Comeau and Boisclair 1998). Echoes weaker than –52 dB were therefore excluded from our analyses. Apart from zooplankton invertebrates, these weak echoes could originate from fish larvae, but our data set does not allow us to verify what they were exactly.

Computations

Fish abundance and distribution were estimated using the number of fish echoes observed per cubic metre of water insonified. The units used to describe this variables is hereafter abbreviated as "fev" (fish echoes per volume of 1 m^3).

The number of echoes recorded within a sector during any given sampling session was estimated using an echo processing system provided by SIMRAD (HydroAcoustic Data Acquisition System, HADAS 4.02; T. Lindem, Department of Physics University of Oslo, Oslo, Norway). HADAS considered signals as single echoes when separated by more than half the pulse length (pulse length = pulse duration × speed of sound in water, 0.22 m for our study). The number of fish echoes was calculated using the number of echoes identified by HADAS as single echoes and the proportion of single fish echoes in our recordings (number of fish echoes = (number of single fish echoes × 100)/proportion of single fish echoes). The proportion of single echoes averaged 70% in recordings obtained at 01:00 and 94% for the other sampling times.

The volume of water insonified in each sector was estimated independently for each sampling session using a modified version of the geometric formula of a cone. The modification applied allowed us to correct our calculation for the volume of the acoustic cone projected above the water surface. This calculation was based on the effective beam angle (11°), the depth of the transducer (0.8 m), and the distance between the transducer and the hydroacoustic shore (computed by HADAS). Estimates of the volume of water insonified ranged from 151 (sector 1) to 244 m^3 (sector 4).

Fish abundance estimates obtained by our approach are interpreted as relative values and will be hereafter abbreviated as "FRA" (fish relative abundance). One fish moving in the acoustic beam, or entering and exiting from it during the 3-min observations, might be insonified more than once and hence generate more than one echo. Moreover, the volume of water insonified was estimated assuming a value of 11° for the effective beam angle. However, in reality, the effective beam angle varies with the TS and the aspect of a fish (lateral or sagittal). Computations were nevertheless made using an effective beam angle of 11° determined using 6 cm TL fish, since these are expected to represent the majority of the targets encountered during our surveys. Finally, the exclusion of echoes weaker than –52 dB from our analyses does not ensure that all signals originating from zooplankton were eliminated. This correction was applied to single as well as to multiple echoes, following our assumption that the proportion of echoes weaker than –52 dB among single echoes equals that of echoes weaker than –52 dB among multiple echoes. However, aggregates of zooplankton may produce multiple echoes that could be included in our analyses if, following the previous assumption, a proportion of multiple echoes is considered to have TS stronger than –52 dB. Multiple echoes from aggregates of invertebrates may exaggerate the proportion of the total voltage attributed to multiple fish echoes and hence diminish the proportion attributed to single fish echoes. Since we used this proportion of single fish echoes to calculate the number of fish echoes, utilization of an erroneous smaller value might cause an overestimation of fish abundance.

Estimates of FRA were computed as individual values for each sector of a given sampling session (FRA_I), as averages over sectors during specific sampling sessions (FRA_S), as averages over sectors and sampling sessions for specific periods of the day (FRA_P during midday, dusk, night, and dawn), and for all combinations of periods of the day and moon phase (FRA_M during new moon, quarter moon, and full moon for specific periods of the day).

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Analyses

Shapiro–Wilk statistics ($W = 0.46$, $p > 0.05$) indicated that FRA_I values were not normally distributed and that they could not be normalized. Comparisons of FRA among time of day, sampling day, moon phase, and month were therefore performed using nonparametric analysis of variance (ANOVA). In these analyses, FRA_I values were ranked and ties were given a mean rank (SAS Institute Inc. 1988). FRA_I estimates of each adjacent sector insonified during a particular sampling session were considered as replicates. Our interpretation of these ANOVA is that even if a portion of the total variation (mean square error term) of FRA_I remains unexplained by the factors we selected and may indeed be caused by unpredictable fish behavior, discrimination of temporal patterns that possibly structure variation of fish abundance will not be prevented.

ANOVA showed that values of FRA obtained at first and last quarters of the moon cycle did not vary significantly for most hours surveyed ($0.11 < p < 0.33$). Only those values obtained at 05:00 or at 21:00 varied significantly among first and last quarter moon ($p < 0.0001$). We believe that the most probable cause of these variations is sun-driven light intensity. Indeed, since timing of sunrise and sunset changed over the summer, luminosity prevailing at 05:00 or at 21:00 varied from one sampling week to another. It is doubtful that, considering the presence of the sun, moon lighting conditions could have caused the patterns we observed. Along the same line of thought, timing of moonrise might be suggested as an explanation of the significant variation among first and last quarters of those FRA values obtained at 05:00 or at 21:00. To test this hypothesis, we need FRA values from all combinations of time of day (05:00 and 21:00), moon phase (both quarters), and timing of moonrise but with constant timing of sunrise and sunset. Since our data set does not comprise such data, we were not able to verify that hypothesis. Nevertheless, the similarity of FRA values obtained at both quarters, coupled with a problem of unequal sample size, led us to pool some data. Indeed, only 1 week of last quarter moon was covered by our sampling schedule, compared with 2 weeks of new, full, and first quarter moon. In addition, sampling during first quarter moon was often prevented due to thunderstorms and heavy rain. Few FRA values were then obtained from either of the two quarters. Hence, first and last quarters were pooled during statistical analyses and are further referred to as data from quarter moon. Furthermore, since a posteriori multiple comparisons among hours of the day indicated no statistically significant difference in FRA at 09:00, 13:00, and 17:00 ($0.1670 < p < 0.5430$), data from these three sampling times were also pooled. These analyses therefore confirmed the validity of our approach to focus on four periods of the day: midday (09:00, 13:00, and 17:00), dusk (21:00), night (01:00), and dawn (05:00).

Simulations

One objective of our work was to develop a sampling strategy that would (*i*) account for expected moon phase effects, (*ii*) maximize the accuracy of fish abundance estimates, and (*iii*) minimize sampling effort. This was achieved by independently simulating FRA_P values for four periods of the day (midday, dusk, night, and dawn) following up to nine different sampling strategies characterized by the identity and the number of moon phases sampled for a total of 30 sets of simulations (Table 1). During the simulations, data were selected in groups of four FRA_I from a specific sampling session. Simulations were implemented using all combinations of groups of four FRA_I that respected the conditions of sampling strategies simulated notwithstanding the sampling day or month. This procedure produced 9–1000 values of FRA_P for each set of simulations. The average and standard deviation of simulated FRA_P were determined for each set of simulations and compared with the corresponding observed FRA_P using percent differences. The influence of the pooling of FRA_I values from first and last quarter moon was verified by supplementary simulations implemented with unpooled FRA_I from both quarters. These simulations were performed for dusk and dawn, the only periods of the day presenting significant variation in FRA among first and last quarters.

Results

Observations

FRA_I ranged from 0 to 70.0 fev and displayed statistically significant variations among consecutive days, months, and moon phase (Table 2). One exception to this rule was midday FRA_I values that did not vary significantly among consecutive days and among moon phase. In addition, FRA_I for the month of July were significantly higher than values obtained in August for all periods of the day except the night (Table 2). While FRA_I varied at all temporal scales explored, our data suggested the existence of structured patterns.

Maximum FRA_S values of up to 51.6 fev were observed at night (74% of the sampling days) or at dusk (26% of the sampling days) (Fig. 2). In contrast, minimum FRA_S values (0 fev) were usually obtained during midday (82%), although few occurred at dawn (12%) and at dusk (6%). FRA_P was lowest during midday (0.1 fev), increased at dusk (3.7 fev), reached a maximum at night (17.0 fev), and decreased at dawn (0.4 fev). The statistical significance of these variations ($F_{5,417} = 94.61$, $p < 0.0001$) confirmed our expectation that, on a daily basis, fish migrated from the littoral to the pelagic zone at night and from the pelagic to the littoral zone at dawn.

Our time series of FRA_S was consistent with the hypothesis that the phase of the moon during hydroacoustic surveys affected estimates of fish abundance (Fig. 3). The effect of the moon phase was most evident at night, with FRA_S values ranging from 7.9 to 46.7 fev during the new moon and from 0.6 to 6.0 fev during the full moon. In contrast, midday FRA_S were unaffected by the moon phase, with values ranging from 0 to 0.7 fev during the new moon and from 0 to 0.9 fev during the full moon. FRA_S obtained during quarter moon nights (0.1–51.6 fev) covered and even surpassed the range of FRA_S observed for the two other moon phases. In summary, our data indicated that fish densities in the pelagic zone on new moon nights ($FRA_M = 21.8$ fev) were, on average, eightfold higher than those observed during full moon ($FRA_M = 2.8$ fev), while quarter moon nights were characterized by higher FRA_M (26.9 fev) and higher variability in fish relative abundance.

Simulations

Simulated FRA_P varied among periods of the day following a pattern similar to that of observed FRA_P (Table 1). Averages of simulated FRA_P during midday (0.1–0.2 fev) increased at dusk (2.4–4.5 fev), reached a maximum at night (2.8–26.6 fev), and decreased at dawn (0.2–0.5 fev). The standard deviation of average FRA_P ranged from 0 to 14.1 and tended to increase with higher FRA_P values. The coefficient of variation of FRA_p ranged from 0 to 200%. The sampling strategy (combination of identity and number of moon phase) had relatively little influence on average simulated FRA_P and its standard deviation for all periods of the day except at night (Table 1). For this period, both the averages

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Table 1. Sampling strategies and results of simulations performed to estimate fish relative abundance (FRA_p) for four periods of the day.

Period of the day	Identity of moon phase sampled	Sampling sessions per moon phase (no.)	Simulated FRA_p values (no.)	Average of simulated FRA_p (fev)	Range of simulated FRA_p (fev)	SD of simulated FRA_p (fev)
Midday	● ○ ☾	1	1000	0.1	0–0.7	0.1
	● ○	1	100	0.1	0–0.8	0.2
	●	2	25	0.1	0–0.6	0.2
	○	2	25	0.2	0–0.5	0.2
	☾	2	25	0.1	0–0.2	0.1
	Random twice		216	0.1	0–0.8	0.2
	Random 3 times		216	0.1	0–0.7	0.1
Dusk	● ○ ☾	1	216	3.6	0.6–7.4	1.3
	● ○	1	36	3.1	0.8–5.9	1.2
	●	2	9	2.4	1.5–4.0	0.9
	○	2	9	3.9	2.1–5.6	1.1
	☾	2	9	4.5	2.0–8.0	1.9
	Random twice		216	3.4	0–8.5	1.7
	Random 3 times		216	3.4	0.6–7.4	1.2
Night	● ○ ☾	1	216	17.1	2.9–34.8	7.8
	● ○	1	36	12.3	4.2–26.4	7.0
	● ☾	1	36	24.2	4.0–49.2	11.8
	○ ☾	1	36	14.7	0.3–28.8	9.6
	●	2	9	21.8	9.9–32.5	7.9
	○	2	9	2.8	0.8–5.0	1.4
	☾	2	9	26.6	5.0–50.2	14.1
	Random twice		216	18.1	0.1–50.2	11.8
	Random 3 times		216	17.4	0.8–45.4	9.9
Dawn	● ○ ☾	1	216	0.4	0–1.0	0.3
	● ○	1	36	0.4	0–0.7	0.2
	●	2	9	0.5	0–1.0	0.3
	○	2	9	0.2	0.2–0.3	0
	☾	2	9	0.5	0–1.2	0.4
	Random twice		216	0.4	0–1.5	0.3
	Random 3 times		216	0.4	0–1.1	0.3

Note: Moon phases sampled for each strategy are new moon (●), full moon (○), and quarter moon (☾). Random twice and three times involve sampling two or three times during a 2-month period (July and August) at randomly selected moon phases. FRA_p estimates are expressed as fish echoes per cubic metre (fev).

Table 2. ANOVA results on temporal variations in fish relative abundance (FRA_t).

	Month			Moon phase			Day		
	df	*F*	*p*	df	*F*	*p*	df	*F*	*p*
Night	1,58	0.10	0.7570	2,58	85.35	0.0001	2,58	4.25	0.0189
Dawn	1,55	13.95	0.0004	2,55	5.33	0.0077	2,55	10.23	0.0002
Midday	1,180	61.03	0.0001	2,180	1.80	0.1682	2,180	2.89	0.0584
Dusk	1,62	196.80	0.0001	2,62	11.38	0.0001	2,62	39.13	0.0001

Note: Monthly effects refer to differences in FRA_t between July and August. Moon phase are new, full, and quarter moon. Day effects represent differences between 3 consecutive days spanning from the day before to the day after a particular moon phase.

and the standard deviations of simulated FRA_p varied by 10-fold depending on the sampling strategy selected. Simulations using data collected during full moon or quarter moon nights provided the lowest (2.8 fev) and highest (26.6 fev) estimates of fish abundance, respectively. Pooling data from first and last quarter moon had negligible effect on the results of our simulations. Indeed, averages of simulated FRA_p produced using either pooled or unpooled data were very similar at dusk (3.6 and 3.9 fev, respectively) and equal at dawn (0.4 fev). Corresponding standard deviations were equal at dusk (1.3 fev) and very similar at dawn (0.3 and 0.2, respectively).

Comparison between observations and simulations

For midday, dusk, and dawn, averages of simulated FRA_p were always within 0–25% of observed values obtained using our original data set. In contrast, simulated FRA_p at night could underestimate, on average, by 84% (sampling

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Fig. 2. Daily variations in fish relative abundance (FRA_S). Estimates for specific sampling sessions are expressed as fish echoes per cubic metre (fev). Lines join FRA_S values observed within a sampling day, and diamonds represent the maximum FRA_S value observed within that day.

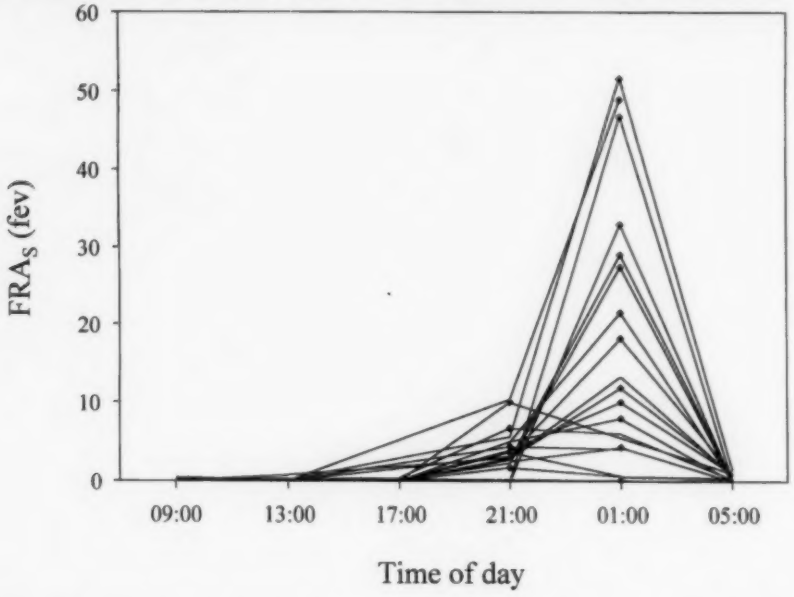
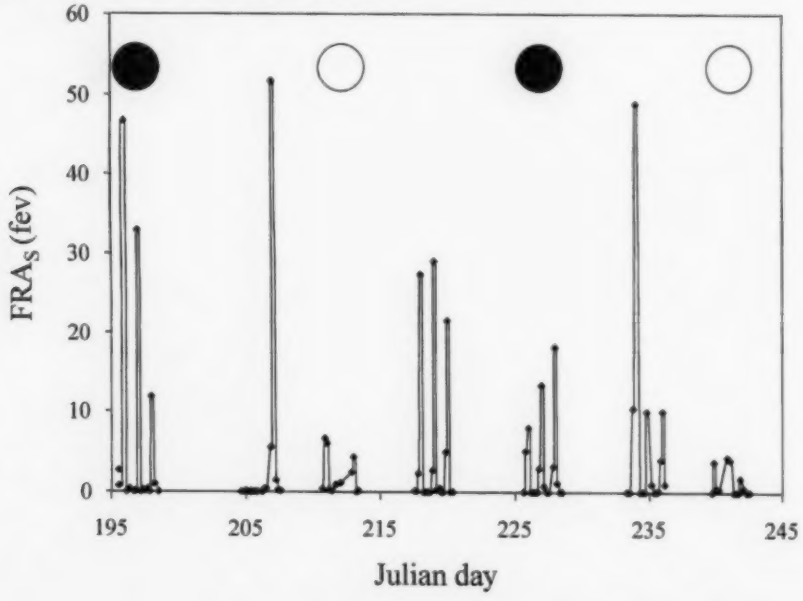


Fig. 3. Time series of estimates of fish relative abundance (FRA_S) obtained during our study. Estimates for specific sampling sessions are expressed as fish echoes per cubic metre (fev). ●, new moon phase; ○, full moon phase.



only during full moon) or overestimate, on average, by 56% (sampling only during quarter moon) the observed FRA_P (Fig. 4). The least consistent sampling strategy (the strategy associated with the highest standard deviation of difference between simulated and observed FRA_P, SD = 83%) was to sample only during quarter moon, while the most consistent sampling strategy was to sample only during full moon nights (SD = 8%). Unfortunately, this strategy consistently underestimated observed FRA_P (Fig. 4). On average, the smallest percent difference between simulated FRA_P and observed FRA_P (3×10^{-5}%) was obtained for the strategy that involved the sampling of one full, one new, and one quarter moon per summer (Fig. 4). This strategy was also the third most consistent approach (SD = 46%, after sampling only

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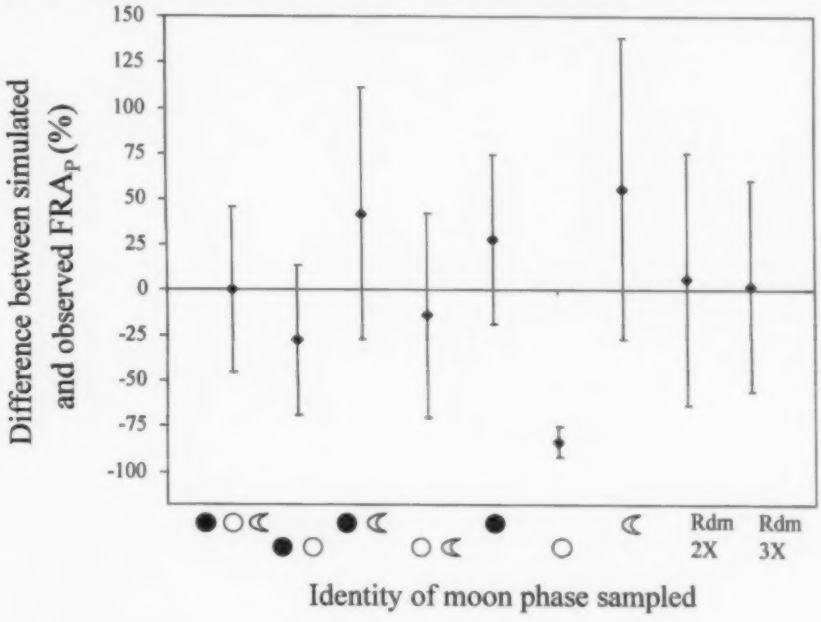
Fig. 4. Comparison between estimates of fish relative abundance (FRA_P) observed at night (01:00) and those simulated for the same period of the day using the nine sampling strategies described in Table 1. Estimates are expressed as fish echoes per cubic metre (fev). Vertical bars represent ±1 SD. ●, new moon phase; ○, full moon phase; ☾, quarter moon phase.



full moon and sampling one full and one new moon) for estimating FRA_P.

Discussion

Our results indicate that the abundance of fish in the pelagic zone of Lake Croche varies among hours of a day, among consecutive days and months, and among successive moon phases. Within-day variations in fish abundance in the pelagic zone of lakes are well established phenomena generally associated with daily vertical (Neverman and Wurtsbaugh 1994; Appenzeller and Leggett 1995; Eckmann and Imbrock 1996) and horizontal migration patterns (Hall et al. 1979; Naud and Magnan 1988; Gauthier and Boisclair 1997). In these studies, 39% (range of 8–82%) of the total number of fish observed in the pelagic zone within 1 day were sampled at night, in contrast with 3% (range of 0–11%) during midday. Our results are consistent with most published studies in terms of both direction (more fish during the night than during the day) and magnitude of the tendencies (night: 71%, range of 12–99%; day: 1%, range of 0–12%). However, an inverse pattern, characterized by higher fish abundance in the pelagic zone during days than during nights, has been described (Baumann and Kitchell 1974; Levy 1990). Regardless of the pattern, daily migrations have been hypothesized to represent strategies to maximize feeding (Baumann and Kitchell 1974; Bohl 1980), minimize predation by piscivorous fish or birds (Gliwicz and Jachner 1992; Eckmann and Imbrock 1996), or minimize metabolic expenditures (Neverman and Wurtsbaugh 1994). Observations of fish and plankton migrations in Lake Croche support the hypothesis that movement of the fish from the littoral towards the pelagic zone at dusk (and back to the littoral zone at dawn) correlates with the peak of zooplankton abundance and biomass observed in the pelagic zone at night (Gauthier and Boisclair 1997). A comparative study of migration patterns in four lakes, including Lake Croche, is also consistent with the hypothesis that the presence of predators may affect fish migration strategy (Gaudreau and Boisclair 1998). We doubt, however, that the minimization of metabolic expenditures may be involved in defining horizontal migrations because this hypothesis requires marked differences in water temperatures experienced by fish in the two habitats.

Day-to-day variation in fish abundance estimates is less frequently documented than daily variation. We observed average variations of threefold between estimates obtained from sampling performed on two consecutive nights and an average coefficient of variation of 53% for sampling on three consecutive nights. Our values are similar to those presented by the few studies that assessed among-day variations. For instance, Comeau and Boisclair (1998) found that fish abundance in the pelagic zone of Lake Croche varied, on average, by twofold between consecutive days. Similarly, Hansson (1993) observed an average coefficient of variation of 27% for estimates of fish abundance obtained from acoustic sampling performed within four nights in Baltic coastal areas. To our knowledge, no hypothesis has been proposed to explain such variations, and our data do not allow us to identify the variables that could be responsible for such day-to-day (or night-to-night) variations. Variations in fish abundance over longer temporal scales, such as months, have been used to estimate fish mortality rates (Beukers and Jones

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1997; Macpherson et al. 1997). While monthly variations are expected, their direction (increase or decrease) and magnitude may represent a compromise between fish mortality rates and recruitment rates of new acoustic targets. New targets may occur through increased population size following reproduction, through changes in fish behavior and distribution (shift from sedentary littoral residents to migratory transient pelagic users), or through changes in fish size (growth of targets initially too small to be recorded by hydroacoustics or to be discriminated from zooplankton). Our study is simplified by the presence of only one fish species, the hybrid of northern redbelly dace and finescale dace, in the pelagic zone during summer months (Gauthier and Boisclair 1998). To conclude that our observation of a 25% decrease in dace relative abundance between July and August corresponds to an index of mortality requires the assumption that a relatively constant number of 0+ dace were available to our acoustic system during surveys. However, the spawning and hatching period of this species extends almost over the complete summer. Moreover, the noise associated with estimates of TS of single fish, as a result of the technological limits of our system and of the effect of fish orientation (lateral or sagittal perspective in the beam of the transducer), does not allow us to discriminate 0+ fish from other components of this population. Although this problem could be solved by using traditional approaches (i.e., trawling) to estimate population age structure, we view our data strictly as indices of the number of fish >4.5 cm TL exploiting the pelagic zone of Lake Croche.

Our study supports the hypothesis that the moon phase affects the number of fish performing horizontal migration at night in Lake Croche. However, the moon phase did not influence estimates of fish abundance obtained during midday. FRA_M during full moon nights was, on average, eightfold lower than during new moon nights. Observations performed during quarter moon nights provided, on average, the highest and most variable values of fish abundance. The effect of the moon phase that we observed is similar to that found by hydroacoustic surveys either for vertical (Luecke and Wurtsbaugh 1993) or horizontal migration (Comeau and Boisclair 1998). The number of captures per unit effort has also been shown to be affected by the moon phase (Collins 1979; Otubusin 1990). However, in these cases, it is difficult to assess if the reduced catch per unit effort is more directly related to a decrease in the number of fish available for fishing than to a reduction of fishing gear efficiency (e.g., due to an increase in the visibility of sampling gears). Hypotheses regarding the mechanism involved in the influence of the moon phase refer to the effect of light on the vulnerability of fish to their predators (Luecke and Wurtsbaugh 1993). The same mechanism has been used to explain variations in zooplankton density following the moon cycle (Gliwicz 1986). During moonlit nights, the open pelagic zone may become too risky for planktivorous fish, since probability of detection by piscivorous fish may increase. Consequently, fewer small fish would migrate to the pelagic zone, since shelter from visual predators could be found in the complex habitat of the littoral zone. In Lake Croche, the hybrid of northern redbelly dace and finescale dace is subject to predation by brook trout and lake trout. However, to confirm the ultimate cause of the patterns that we observed, we would have to perform our observations in more than one lake and assess the relative influence of other factors. In addition, the specific data that we collected suggest that factors other than light intensity may define the effect of the moon phase. The nights of Julian days 211 and 213 coincided with a full moon. The percent cloudiness for the night of Julian day 211 was approximately 50% compared with 100% for the night of Julian day 213. Despite this situation, FRA was lower on the night that was 100% covered by clouds (4.3 compared with 6.0 fev). Similarly, at the following full moon, more fish migrated during the clear night of Julian day 241 (0% cloudiness) than during the partly covered night (60% cloudiness) of Julian day 242 (4.0 compared with 0.7 fev). Furthermore, we found no significant difference in FRA estimated on full moon nights characterized by different cloud cover ($F_{2,16} = 1.35$, $p = 0.2861$). Hence, even if light intensity was used to predict the migratory behavior of fish, our results show that it is not the only factor determining this behavior.

Examination of the factors that cause fish relative abundance (FRA) in the pelagic zone to vary at different temporal scales (among hours, days, months, and moon phase) suggests that FRA values vary most among different periods of the day (particularly between day and night) and among different moon phases (particularly between full and new moon). This situation represents an advantage, since it allows the development of sampling strategies based on predictable and recurrent patterns (time of day and moon phase). Our simulations indicated that two of the nine strategies tested produced equally accurate estimates of seasonal mean FRA. These strategies performed better than the others for all periods of the day. Both consisted in sampling a lake on three occasions over a 2-month period. One strategy was more systematic and required hydroacoustic sampling of lakes once at a full moon, once at a quarter moon, and once at a new moon. The other required sampling on 3 days selected at random. However, because of daily and lunar variations observed in many studies, including ours, assessment of the mean abundance of zooplanktivorous fish in the pelagic zone of a lake may require hydroacoustic surveys performed during daytime and nighttime on a sufficient number of dates to adequately represent lunar variations. Because the results of our simulations depend on our particular data set, we cannot affirm that the strategies that we propose are appropriate for large pelagic piscivorous fish. This hypothesis could not be verified with statistical analyses because echoes greater than –40dB represented only 15% of our signals. We cannot confirm either that the strategies that we offer would be applicable to other lake communities. In addition, sampling protocols always depend on the purpose of surveys. For instance, sampling only new moon nights may be sufficient to estimate mortality rates of well-defined nocturnal and pelagic feeders. If we exclude very tight sampling schedules (every day at daytime and nighttime), there may not be a single best sampling strategy. In this context, the contribution of our analyses may be to provide indications of the magnitude of variations found at different temporal scales and of the potential biases induced by insufficiently rigorous sampling strategies. This information may represent a framework for developing rational hydroacoustic sampling protocols.

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References

Appenzeller, A.R., and Leggett, W.C. 1995. An evaluation of light-mediated vertical migration of fish based on hydroacoustic analysis of the diel vertical movements of rainbow smelt (*Osmerus mordax*). Can. J. Fish. Aquat. Sci. **52**: 504–511.

Austin, R.H., Phillips, B.F., and Webb, D.J. 1976. A method for calculating moonlight illuminance at the Earth's surface. J. Appl. Ecol. **13**: 741–748.

Baumann, P.C., and Kitchell, J.F. 1974. Diel patterns of distribution and feeding of bluegill (*Lepomis macrochirus*) in Lake Wingra, Wisconsin. Trans. Am. Fish. Soc. **2**: 255–260.

Begg, G.W. 1976. The relationship between the diurnal movements of some of the zooplankton and the sardine *Limnothrissa miodon* in Lake Kariba, Rhodesia. Limnol. Oceanogr. **21**: 529–539.

Beukers, J.S., and Jones, G.P. 1997. Habitat complexity modifies the impact of piscivores on a coral reef fish population. Oecologia, **114**: 50–59.

Bohl, E. 1980. Diel pattern of pelagic distribution and feeding in planktivorous fish. Oecologia, **44**: 368–375.

Collins, J.J. 1979. Relative efficiency of multifilament and monofilament nylon gill net towards lake whitefish (*Coregonus clupeaformis*) in Lake Huron. J. Fish. Res. Board Can. **36**: 1180–1185.

Comeau, S., and Boisclair, D. 1998. Day-to-day variation in fish horizontal migration and its potential consequence on estimates of trophic interactions in lakes. Fish. Res. **35**: 75–81.

Eckmann, R., and Imbrock, F. 1996. Distribution and diel vertical migration of Eurasian perch (*Perca fluviatilis* L) during winter. Ann. Zool. Fenn. **33**: 679–686.

Gaudreau, N., and Boisclair, D. 1998. The influence of spatial heterogeneity on the study of fish horizontal daily migration. Fish. Res. **35**: 65–73.

Gauthier, S., and Boisclair, D. 1997. The energetic implications of diel onshore–offshore migration by dace (*Phoxinus eos* × *P. neogaeus*) in a small oligotrophic lake. Can. J. Fish. Aquat. Sci. **54**: 1996–2006.

Gauthier, S., Boisclair, D., and Legendre, P. 1997. Evaluation of a variable angle scanning method to estimate relative abundance and distribution of fish using a single-beam echosounder in shallow lakes. J. Fish Biol. **50**: 208–221.

Gliwicz, Z.M. 1986. A lunar cycle in zooplankton. Ecology, **67**: 883–897.

Gliwicz, Z.M., and Jachner, A. 1992. Diel migrations of juvenile fish: a ghost of predation past or present? Arch. Hydrobiol. **124**: 385–410.

Hall, D.J., Werner, E.E., Gilliam, J.F., Mittelbach, G.G., Howard, D., Doner, C.G., Dickerman, J.A., and Stewart, A.J. 1979. Diel foraging behavior and prey selection in the golden shiner (*Notemigonus crysoleucas*). J. Fish. Res. Board Can. **36**: 1029–1039.

Hansson, S. 1993. Variation in hydroacoustic abundance of pelagic fish. Fish Res. **16**: 203–222.

Hilborn, R., and Walters, C.J. 1992. Quantitative fisheries stock assessment: choice, dynamics and uncertainty. Chapman and Hall, London, U.K.

Imbrock, F., Appenzeller, A., and Eckmann, R. 1996. Diel and seasonal distribution of perch in Lake Constance: a hydroacoustic study and in situ observations. J. Fish Biol. **49**: 1–13.

Jeppesen, E., Lauridsen, T., Mitchell, S.F., and Burns, C.W. 1997. Do planktivorous fish structure the zooplankton communities in New Zealand lakes? N.Z. J. Mar. Freshwater Res. **31**: 163–173.

Johannes, M.R.S. 1993. Prey aggregation is correlated with increased predation pressure in lake fish communities. Can. J. Fish. Aquat. Sci. **50**: 66–73.

Kubecka, J. 1994. Simple model on the relationship between fish acoustical target strength and aspect for high-frequency sonar in shallow waters. J. Appl. Ichthyol. **10**: 75–81.

Kubecka, J. 1996. Use of horizontal dual-beam sonar for fish surveys in shallow waters. *In* Stock assessment in inland fisheries. *Edited by* I.G. Cowx. Fishing News Books, Blackwell, Oxford, U.K. pp. 165–178.

Levy, D.A. 1987. Review of the ecological significance of diel vertical migrations by juvenile sockeye salmon. (*Oncorhynchus nerka*). *In* Sockeye salmon (*Oncorhynchus nerka*) population biology and future management. *Edited by* H.D. Smith, L. Margolis, and C.C. Wood. Can. Spec. Publ. Fish. Aquat. Sci. No. 96. pp. 44–52.

Levy, D.A. 1990. Reciprocal diel vertical migration behavior in planktivores and zooplankton in British Columbia lakes. Can. J. Fish. Aquat. Sci. **47**: 1755–1764.

Lucas, M.C., Mercer, T., Batley, E., Frear, P.A., Peirson, G., Duncan, A., and Kubecka, J. 1998. Spatio-temporal variations in the distribution and abundance of fish in the Yorkshire Ouse system. Sci. Total Environ. **210/211**: 437–455.

Luecke, C., and Wurtsbaugh, W.A. 1993. Effects of moonlight and daylight on hydroacoustic estimates of pelagic fish abundance. Trans. Am. Fish. Soc. **122**: 112–120.

MacLennan, D.N., and Simmonds, E.J. 1992. Fisheries acoustics. Chapman and Hall, London, U.K.

Macpherson, E., Biagi, F., Francour, P., Garcia-Rubies, A., Harmelin, J., Harmelin-Vivien, M., Jouvenel, J.Y., Planes, S., Vigliola, L., and Tunesi, L. 1997. Mortality of juvenile fishes of the genus *Diplodus* in protected and unprotected areas in the western Mediterranean Sea. Mar. Ecol. Prog. Ser. **160**: 135–147.

Mills, E.L., Forney, J.L., and Wagner, K.J. 1987. Fish predation and its cascading effect on the Oneida Lake food chain. *In* Predation: direct and indirect impacts on aquatic communities. *Edited by* W.C. Kerfoot and A. Sih. University Press of New England, Hanover, N.H. pp. 118–132.

Misund, O.A. 1997. Underwater acoustics in marine fisheries and fisheries research. Rev. Fish Biol. Fish. **7**: 1–34.

Naud, M., and Magnan, P. 1988. Diel onshore-offshore migrations in northern redbelly dace, *Phoxinus eos* (Cope), in relation to prey distribution in a small oligotrophic lake. Can. J. Zool. **66**: 1249–1253.

Neverman, D., and Wurtsbaugh, W.A. 1994. The thermoregulatory function of diel vertical migration for a juvenile fish, *Cottus extensus*. Oecologia, **98**: 247–256.

Otubusin, S.O. 1990. Effects of lunar periods and some other parameters on fish catch in Lake Kainji, Nigeria. Fish. Res. **8**: 233–245.

Rose, G.A. 1992. A review of problems and new directions in the

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application of fisheries acoustics on the Canadian East Coast. Fish. Res. **14**: 105–128.

SAS Institute Inc. 1988. SAS/STAT user's guide, release 6.03 edition. SAS Institute Inc., Cary, N.C.

Schindler, D.E., Carpenter, S.R., Cottingham, X.H., Hodgson, J.R., Kitchell, J.F., and Soranno, P.A. 1996. Food web structure and littoral zone coupling to pelagic trophic cascades. *In* Food webs: integration of patterns and dynamics. *Edited by* G.A. Polis and K.O. Winemiller. Chapman & Hall, NewYork. pp. 96–105.

Vanni, M.J. 1996. Nutrient transport and recycling by consumers in lake food webs: implications for algal communities. *In* Food webs: integration of patterns and dynamics. *Edited by* G.A. Polis and K.O. Winemiller. Chapman & Hall, NewYork. pp. 81–95.

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Interactions between zebra mussels (*Dreissena polymorpha*) and microbial communities

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Abstract: Zebra mussels (*Dreissena polymorpha*) have had an enormous impact on aquatic environments. However, little is known concerning their interactions with microbial communities. In these studies, the ability of zebra mussels to derive nutrition from bacterioplankton and their effect on microbial community diversity were investigated in samples from the Hudson River, New York, and in laboratory studies. Clear physiological responses to starvation were observed, including decreases in respiration rates, lipid content, and total weight, that were reversed after feeding zebra mussels a diet of bacteria. Clearance rates of bacteria were correlated with bacteria size ($r^2 = 0.995$), with the lowest clearance rates associated with small indigenous river bacteria (size = 0.03 ± 0.04 μm^3, clearance rate = 0.08 ± 0.02 mL·mussel^{-1}·min^{-1}). Comparison of the diversity of microbial communities in zebra mussel tissue extract, detritus, and pseudofecal material associated with zebra mussel colonies, surrounding water, and sediment samples revealed distinct microbial assemblages associated with these environments. The overall ecological effect and importance of bacteria – zebra mussel interactions remains unclear, but these studies indicate that these interactions occur and should be included in our efforts to better understand the impact of zebra mussels on aquatic systems.

Résumé : Les moules zébrées (*Dreissena polymorpha*) ont un impact énorme sur les milieux aquatiques, pourtant on connaît très peu leurs interactions avec les communautés microbiennes. Nous avons étudié la capacité des moules zébrées de se nourrir de bactérioplancton et leurs effets sur la diversité des communautés microbiennes, à partir d'échantillons prélevés dans l'Hudson (New York), et en laboratoire. Des réactions physiologiques évidentes à l'inanition ont été observées, notamment la baisse du taux de respiration, du taux de lipides et du poids total; ces effets ont été inversés quand on a nourri les moules zébrées de bactéries. Nous avons corrélé le taux d'élimination des bactéries et la taille des bactéries ($r^2 = 0,995$), les plus faibles taux d'élimination étant associés aux petites bactéries indigènes de rivière (taille = 0,03 ± 0,04 μm^3, taux d'élimination = 0,08 ± 0,02 mL·moule^{-1}·min^{-1}). Nous avons étudié les assemblages microbiens des extraits de tissus, des détritus et des pseudofèces de différentes colonies de moules zébrées, et ceux des échantillons de sédiments et de l'eau aux environs de ces colonies; nous avons observé que les assemblages étaient différents selon le milieu. L'impact et l'importance écologiques des interactions bactéries – moules zébrées restent flous, mais nos expériences font ressortir que ces interactions existent et qu'elles doivent entrer dans les travaux menés pour mieux comprendre l'impact des moules zébrées sur les milieux aquatiques.

[Traduit par la Rédaction]

Introduction

Since their first introduction into North America in 1985 or 1986 (Roberts 1990; Carlton 1993; Ludyanskiy et al. 1993), the zebra mussel (*Dreissena polymorpha*) and the quagga mussel (*Dreissena bugensis*) have quickly spread throughout U.S. and Canadian waterways and lakes. Despite efforts to control the spread of zebra mussels, extensive populations are now permanently established in North American aquatic systems. In North America, the current range of the zebra mussel extends north to Canada, south to the Gulf of Mexico in the Mississippi River, as far west as Oklahoma, and in the east to Kingston, N.Y.

In addition to the nuisance caused by zebra mussels related to biofouling, the introduction of zebra mussels into North American aquatic systems has had profound ecological impacts. Most significantly, it has been demonstrated that zebra mussels impact phytoplankton production and standing crops, nutrient and contaminant cycling, and indigenous macroscopic flora and fauna (MacIsaac et al. 1992; Holland 1993; Leach 1993; Nicholls and Hopkins 1993; Bruner et al. 1994; Holland et al. 1995; Caraco et al. 1997; Karatayev et al. 1997; Strayer et al. 1999). However, little attention has been focused on the relationships between zebra mussels and microbial ecosystem components, despite evidence that phytoplankton food resources are often limiting for zebra mussel populations and that zebra mussels may be capable of capturing bacteria-sized particles.

Zebra mussels may interact either directly or indirectly

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with microbial populations by utilizing bacteria as a food source (direct interaction) or indirectly by influencing the composition, activity, and growth of microbial communities. Although several studies have demonstrated that zebra mussels can capture and ingest bacteria (Silverman et al. 1995; Lei et al. 1996; Roditi et al. 1996), because these studies were conducted over short time periods, growth of zebra mussels due to the consumption of bacteria has not been demonstrated.

Alternatively, the presence of zebra mussels and the large quantities of fecal and pseudofecal material that they produce may significantly affect microbial populations and microbial-mediated processes by enhancing bacterial growth. Recent observations made in the Hudson River, New York, suggest that there is increased total bacterial biomass and heterotrophic activity associated with zebra mussel colonies compared with surface sediments (Findlay et al. 1998; Strayer et al. 1999), but only a few studies have addressed these indirect effects of zebra mussels on bacterial populations (Cotner et al. 1995; Findlay et al. 1998), and none, to our knowledge, have examined the effect on microbial community structure.

In this study, we report on the results of laboratory experiments and field observations that indicate that zebra mussels can sustain positive growth by ingestion of bacteria, and we describe initial studies suggesting that the presence of zebra mussels influences the composition of microbial populations.

Methods

Sample collection

All samples (zebra mussels, water, sediment, and the material trapped between zebra mussels) were collected from the Hudson River near the Troy lock and dam (river kilometre 248) near Troy, N.Y., at low tide. Material thought to consist of trapped detritus, sediment, and pseudofecal matter was sampled using a sterile 60-cm^3 syringe without a needle. The syringe was held in close proximity to exposed zebra mussel colonies (approximately 50 cm below the surface) and the material trapped between animals was gently collected into the syringe. For the purpose of discussion here, this material is simply referred to as pseudofecal matter (PFM). Water was also collected using a sterile 60-cm^3 syringe that was held approximately 10 cm below the water surface above zebra mussel colonies. Freshly collected PFM or water was transferred to sterile 50-cm^3 tubes and formaldehyde fixed (3.7% final concentration) in the field. Surface sediment samples (approximately 50 g) adjacent to zebra mussel colonies were collected using a sterile spatula, transported to the laboratoty, and replicate 1-g samples fixed in 9 mL of 3.7% formaldehyde. Zebra mussels for microbial analysis were collected and surface sterilized using a 5% bleach solution, as previously described for microbiological analysis of bivalve tissues (Regan et al. 1993). This treatment did not kill the zebra mussels. After collection, zebra mussels were transported live to the laboratory and the tissue was removed, weighed, homogenized, and preserved in two volumes of 3.7% formaldehyde. All samples were stored at 4°C until analysis.

For starvation and feeding studies, living adults ranging in size from 0.5 to 1.5 cm were gently removed from submerged rocks using a small spatula, placed in buckets containing river water, and transported to the laboratory within 1 h of collection. Living animals were maintained at 13 ± 2°C in nonsterile artificial river water prepared with distilled water (0.5 mM NaCl, 0.4 mM $CaCl_2$, 0.2 mM $NaHCO_3$, 0.05 mM KCl, 0.2 mM $MgSO_4$; Dietz et al. 1994) and treated as described below.

Starvation and starvation recovery studies

Zebra mussels were starved for 3 months by maintaining them in artificial river water without feeding. These studies were conducted in small (1 L) aerated aquaria maintained at 13 ± 2°C. Approximately 100 animals were maintained in each aquaria. At monthly intervals, 10 animals were removed for analysis of respiration rates, lipid content, and total dry weight. Each analysis was at a minimum triplicated. After a 3-month starvation period, when a clear physiological response had been observed experimentally, feeding studies were initiated. Water in each aquarium was replaced weekly. Each experiment was at a minimum triplicated. Starved zebra mussels were fed an estuarine *Pseudomonas* isolate (strain WJT-1C; Frischer et al. 1996*a*). Overnight cultures of bacteria were grown in sterile artificial river water supplemented with peptone (5 $g \cdot L^{-1}$) and yeast extract (1 $g \cdot L^{-1}$) at 28°C. Cells were washed by centrifugation in artificial river water (no nutrients) and concentrated 100-fold in fresh sterile artificial river water. Final cell concentrations were determined by direct DAPI counting (Kepner and Pratt 1994). Cell concentrates contained approximately 10^{11} cells$\cdot mL^{-1}$. Cells were stored at 4°C until use but were never stored longer than 2 weeks.

Zebra mussels were fed at three bacterial concentrations such that after each feeding, the concentration of bacteria in each experimental aquarium was 10^6, 10^8, or 10^9 cells$\cdot mL^{-1}$. Each aquarium contained 500 mL of artificial river water and 10 zebra mussels. Zebra mussels fed with 10^6 and 10^8 cells were fed approximately three times per week for a total of 10 feedings (10^9 and 10^{11} cells total per aquarium). Thus, each zebra mussel (10 per aquarium) in these treatments was exposed to 5×10^8 and 5×10^{10} cells, respectively, during these experiments. Zebra mussels that were fed at the level of 10^9 cells$\cdot mL^{-1}$ were only fed once per week for a total of four feedings, since at this concentration, it took a full week to clear the bacteria. Thus, each of these zebra mussels were exposed to a total of 2×10^{11} cells in a month. All the zebra mussels used in these studies survived for the duration of the experiment. The effects of feeding bacteria on respiration rates, lipid content, and weight were compared relative to unfed controls over a 1-month period. These studies were carried out at a constant temperature of 13 ± 2°C.

Dry weight

The dry tissue weight of each animal was determined by removing the animal from its shell, drying at 105°C for 48 h, and weighing. For each weight determination, the average weight of 10 zebra mussels was determined and normalized to shell length.

Lipid content

Total lipid was determined using a gravimetric method (Barnes and Blackstock 1973). Dried tissue from five animals was ground, weighed, and homogenized with 5 mL of a 2:1 (v/v) chloroform–methanol mixture. The homogenate was filtered through a Whatman No. 1 filter. Lipids were purified by organic extraction in a separatory funnel after the addition of 4 mL of 0.9% aqueous NaCl. Beaker solvent contents were evaporated and lipid extracts weighed.

Oxygen uptake

Determination of oxygen uptake rates (V_{O_2}) was accomplished using a YSI model 5300 biological oxygen monitor with a sampling apparatus consisting of a temperature-controlled sealed sample chamber with circulating water. The total volume of the chambers and connecting tubes was 71 mL. Zebra mussels were placed in the sampling chamber and oxygen consumption rates measured for 10 min after an initial 10-min calibration period (without zebra mussels at 100% oxygen saturation). Oxygen con-

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sumption rates were calculated by linear regression as the drop in P_{O_2}.

Clearance rate

The effect of bacteria size on clearance rates by zebra mussels was determined by feeding zebra mussels with bacteria of different sizes. Bacterial strains used in these studies included *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas* sp. WJT-1C, and indigenous bacteria from the Hudson River. *Bacillus subtilis* and *E. coli* were grown in LB (10 g tryptone·L^{-1}, 5 g yeast extract·L^{-1}, 10 g NaCl·L^{-1}) at 28 and 37°C, respectively. For strain descriptions, see Frischer et al. (1996*a*, 1996*b*). Cell concentration was determined by direct epifluorescence counting after staining with DAPI. Cell enumeration and size determination were facilitated using an imaging system as previously described by Verity and Sieracki (1993) with contemporary upgrades including a cooled three-chip color-integrating CCD (Photonics Science Color CoolView) and Image Pro Plus version 2.0 for Windows 95.

Clearance rate was determined from particle depletion studies and was defined as the volume of water from which a zebra mussel has removed all particles per unit of time. Experiments were conducted at a constant temperature of 13°C. Bacteria were added to fresh artificial river water (1 L) to give a final concentration of 10^5–10^6 cells·mL^{-1}. Bacterial cell densities of Hudson River water were approximately 10^5 cells·mL^{-1}. For each experiment, two 500-mL aquaria were established. Ten zebra mussels were placed into one aquarium, and the other aquarium remained zebra mussel free as a control. Water was sampled (1 mL) at 15-min intervals for 1 h. Clearance rates were calculated as previously described (Coughlan 1969; Horgan and Mills 1997), except that bacteria settling was accounted for using zebra mussel free control treatments with the following formula:

$$m = M/nt[(\ln C_0 - \ln C_t) - (\ln C_{0C} - \ln C_{tC})]$$

where m is the individual particle clearance rate (millilitres per zebra mussel per minute), M is the total volume in the container, n is the number of zebra mussels, t is the time at which the sample was taken, C_0 is the concentration of bacteria at time zero, C_t is the concentration of bacteria at time t, and C_{0C} and C_{tC} are the initial and final bacterial cell concentrations of the zebra mussel free control treatments. Final clearance rates were normalized to shell length.

Microbial diversity

A suite of phylogenetic group-specific 16S rRNA targeted oligonucleotide probes in conjunction with an in situ whole-cell hybridization protocol were utilized to describe metabolically active microbial communities associated with zebra mussels, sediments, water, and PFM. Metabolically active bacteria were operationally defined as cells with sufficient ribosomal content to be detected by hybridization with a single 16S rRNA targeted fluorescently labeled oligonucleotide probe (Williams et al. 1998). Probes differentiated between the Flexibacter/Cytophaga/Bacterioides phylum (Flavo), the Alpha, Beta, Gamma, and Delta subdivisions of the proteobacteria, and the High and Low G+C subdivisions of the Gram-positive phylum. All of these probes were previously developed and have been described elsewhere (Frischer et al. 1996*b*; unpublished data). Three universal probes were combined (Primer A and Primer C (Lane et al. 1985) and Univ 342 (Vescio and Nierzwicki-Bauer 1993)) and utilized to enumerate the total number of metabolically active bacteria in all samples. All oligonucleotide probes were labeled with tetramethylrhodamine 5/-6 (TRITC) (Molecular Probes, Eugene, Oreg.) by means of a 5′ amino terminus linker and visualized as red-fluorescing cells under green excitation, as previously described (Braun-Howland et al. 1992; Frischer et al. 1996*b*). Total bacterial cell counts were determined by epifluorescence counting after DAPI staining (Kepner and Pratt 1994) as described above. The in situ hybridization procedure, used in conjunction with epifluorescence microscopy, was essentially as previously described (Braun-Howland et al. 1992) with modifications necessary for water samples (Williams et al. 1998). In brief, formaldehyde-fixed samples were permeabilized and dehydrated in ethanol–formaldehyde (90:10) for 1 h, filtered onto a 0.22-μm polycarbonate filter, and washed three times with 1 mL of 1× SET (150 mM NaCl, 20 mM Tris–HCl, pH 7.8, 1 mM EDTA). The filter was then stained with 1 mL of sterile-filtered 0.02% Igalan black (0.2 g 1% acetic acid·100 mL^{-1}) and rewashed three times with 1 mL of 1× SET, mounted on a glass slide, overlaid with oligonucleotide probe mixture (340 ng·mL^{-1}) plus DAPI (3 μg·mL^{-1}) in hybridization solution (0.2% w/v bovine serum albumin, 0.01% w/v polyadenylic acid, 5× SET, 11% w/v dextran sulfate), and hybridized in a humidified chamber at 37°C overnight. Filters were washed for 1 h in three changes of 1× SET, air dried, and mounted with 50% glycerol in 1× SET under a cover glass. Total cell counts (DAPI stained) were visualized using a wide UV filter set (U-M536), with exciter filter BP 330–385, dichroic mirror DM 400, and barrier filter BA 420. Metabolically active cells (universal probe) and phylogenetically identified cells (group-specific probes) were enumerated using a green filter set (U-M526), with exciter filter 510–550, dichroic mirror DM 570, and a barrier filter BA 590. An Olympus BX-60 fluorescence microscope equipped with a 100× UPLANFL NA 1.3 oil objective was used.

Data analysis

ANOVA and t tests were used to compare the physiological responses of zebra mussels to starvation and bacterial feeding after confirming data normality and equivalence of variances. The relationship between clearance rate and bacteria size was determined by linear regression analysis. Data normality, variance equivalency, and regression analysis were facilitated using the algorithms provided in the Jandel SigmaStat statistics package (version 1.0) (Jandel Scientific Software, San Rafael, Calif.). Microbial diversity and group dominance were quantified and ranked using the Shannon–Wiener function after all group-specific bacterial counts were normalized as a percentage of the total community (Krebs 1994). Diversity indexes were compared by ANOVA.

Results and discussion

Bacteria as food

In these studies, we investigated the ability of zebra mussels to utilize bacteria as a sole food source over a 1-month period. Initially, zebra mussels were starved and physiological responses to starvation, including respiration rate, lipid content, and total dry weight, were documented. After clear starvation responses were observed (3 months), zebra mussels were fed cultured bacteria and recovery from starvation was monitored. Feeding studies were conducted after starvation to simulate zebra mussels that were growth limited by the availability of fresh phytoplankton. These conditions are believed to resemble early-spring or late-fall conditions. Recovery from a starvation state was interpreted in these studies as indicating that zebra mussels could utilize bacteria as a source of nutrition. Zebra mussels that were maintained in the laboratory and starved for 3 months showed significant decreases in basal metabolic rates, lipid content, and weight. Over the 3-month starvation period, basal metabolic rate (oxygen consumption) decreased significantly ($p < 0.0001$) from 2.35 ± 0.02 μL O_2·mm shell length^{-1}·h^{-1} ($n = 21$) at the start of the experiments to 1.32 ± 0.1 μL O_2·mm shell

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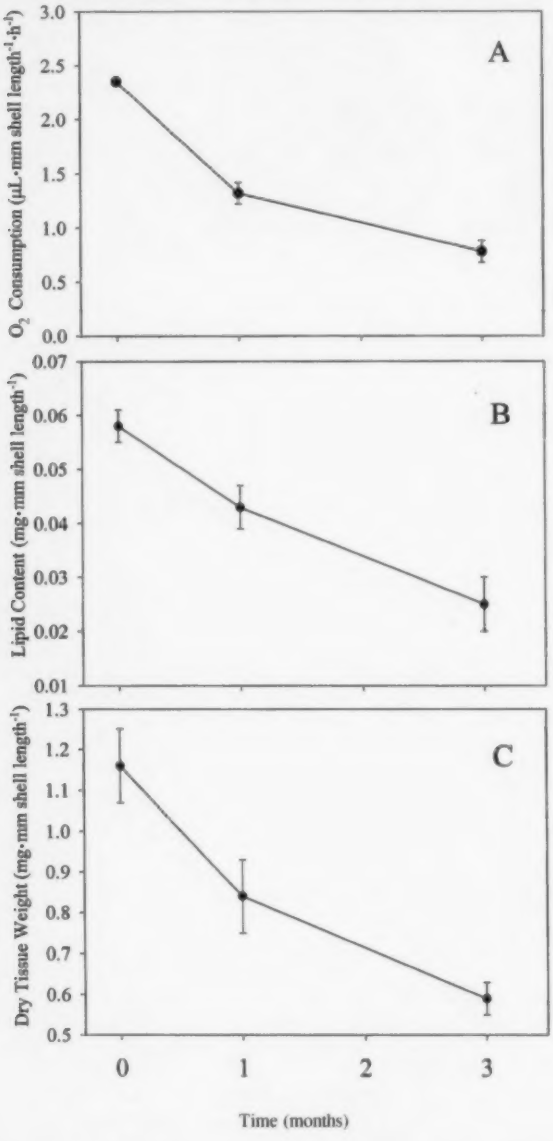
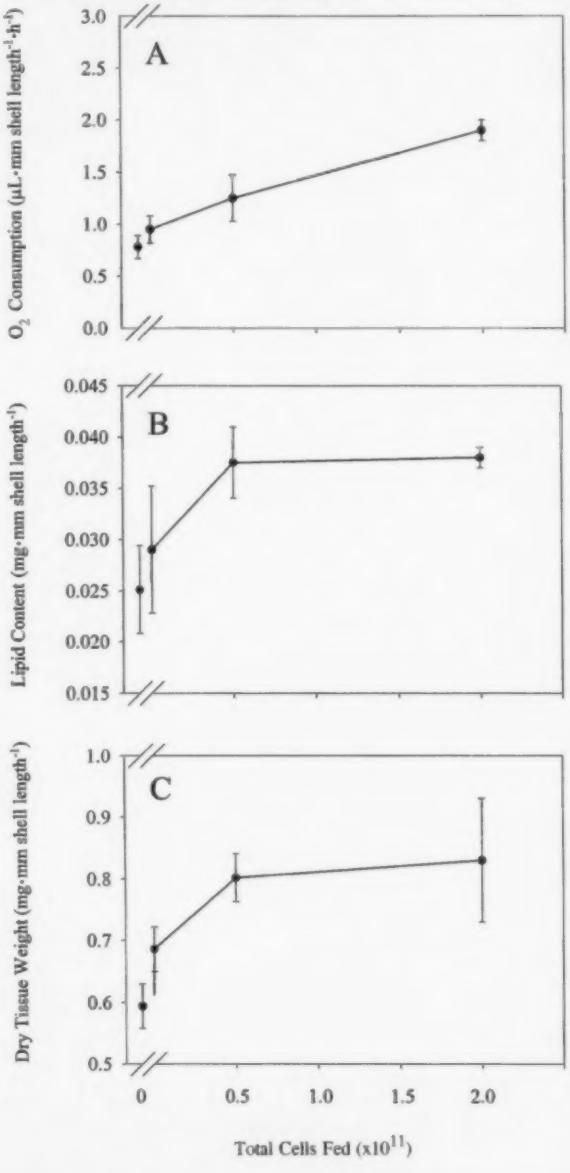
Fig. 1. Physiological effects of starvation of zebra mussels over a 3-month period on (A) respiration rates, (B) lipid content, and (C) total dry weight. Error bars represent 1 SD of the mean.



length^{-1}·h^{-1} (n = 20) after 1 month of starvation and to 0.78 ± 0.1 µL O_2·mm shell length^{-1}·h^{-1} (n = 7) after 3 months of starvation (Fig. 1A). Lipid content also decreased significantly during this starvation period (p < 0.0002) from 0.058 ± 0.003 to 0.023 ± 0.005 mg·mm shell length^{-1} (Fig. 1B). Similarly, zebra mussel tissue dry weight decreased significantly (p < 0.0072) after starvation from 1.16 ± 0.09 to 0.59 ± 0.04 mg·mm shell length^{-1} (Fig. 1C). Glycogen concentrations in zebra mussels were also determined in initial experiments. However, there was no significant correlation between glycogen concentrations and starvation, so glycogen determinations were not continued

Fig. 2. Effect of feeding starved zebra mussels for 1 month with a bacterial culture (*Pseudomonas* sp.) (A) on respiration rates, (B) lipid content, and (C) total dry weight. Zebra mussels were fed at concentrations of 0, 10^6, 10^8, and 10^9 cells·mL^{-1} resulting in exposure to 0, 5.0×10^8, 5.0×10^{10}, and 2.0×10^{11} cells·zebra mussel^{-1}, respectively, over the 1-month feeding period. Error bars represent 1 SD of the mean.



throughout the study (data not shown). Thus, based on oxygen consumption rates, lipid content, and total dry weight, a clear physiological response to starvation was observed.

Recovery from starvation was proportional to the amount of bacteria fed during the feeding period. Zebra mussels exposed during the feeding portion of the experiments to 2×10^{11}, 5×10^{10}, 5×10^8, and no cells, respectively, recov-

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ered with respect to respiration rates before starvation (2.35 μL O_2·mm shell length^{-1}·h^{-1}) and after 3 months of starvation (0.78 μL O_2·mm shell length^{-1}·h^{-1}) by 71.3% (1.9 μL O_2 ·mm shell length^{-1}·h^{-1}), 29.9% (1.25 μL O_2·mm shell length^{-1}·h^{-1}), 10.8% (0.95 μL O_2·mm shell length^{-1}·h^{-1}), and 0% (0.0 μL O_2·mm shell length^{-1}·h^{-1}) (Fig. 2A). Recovery, as indicated by lipid content, also exhibited a response proportional to the amount of bacteria fed relative to prestarvation (0.058 mg·mm shell length^{-1}) and poststarvation values (0.025 mg·mm shell length^{-1}). Animals that were exposed to the highest amount of bacteria (2×10^{11} cells) recovered 39.1% (0.038 mg·mm shell length^{-1}) of the total lipid content relative to prestarvation conditions, those exposed to 5×10^{10} cells recovered by 37.6% (0.0375 mg·mm shell length^{-1}), and animals exposed to 5×10^{8} cells recovered by 11.8% (0.029 mg·mm shell length^{-1}). No increase in lipid content was observed in control treatments when bacteria were not fed (Fig. 2B). Similarly, total weight recovery was proportional to feeding levels relative to prestarvation (1.16 mg·mm shell length^{-1}) and poststarvation values (0.59 mg·mm shell length^{-1}). Weight recoveries were 41.4% (0.83 mg·mm shell length^{-1}), 36.5% (0.802 mg·mm shell length^{-1}), 16.1% (0.686 mg·mm shell length^{-1}), and 0% (0.0 mg·mm shell length^{-1}) for animals fed 2×10^{11}, 5×10^{10}, 5×10^{8}, and no cells, respectively (Fig. 2C).

Because a recovery from starvation conditions was observed after feeding bacteria, the results demonstrated that zebra mussels were able to utilize cultured bacteria as a food source. Previous studies have reported the ability of zebra mussels to capture and or utilize bacteria over shorter time periods of minutes to hours (Cotner et al. 1995; Silverman et al. 1995; Lei et al. 1996; Roditi et al. 1996; Horgan and Mills 1997), but to our knowledge, this is the first report showing that zebra mussels can sustain growth on a sole diet of bacteria over a more realistic growth period (weeks to months).

The assimilation efficiency of bacterial biomass by zebra mussels was estimated as the ratio of the amount of bacterial weight fed to the zebra mussels (estimated as 8×10^{-13} g·cell^{-1} for cultured bacteria; Fagerbakke et al. 1996) to the amount of weight (dry) gained by the zebra mussels during the feeding period. By this estimation, the efficiency of assimilation was calculated as 0.15–22.5% depending on the concentration of cells that were fed. Lower assimilation efficiencies were observed at the higher feeding concentrations, indicating that zebra mussel filtration of bacteria was saturated. These values represent a conservative estimation of assimilation efficiency, since they do not take into account energy utilized for respiration or metabolic losses (e.g., feces production). Assimilation efficiencies calculated from these studies are similar to previously reported values derived from short-term radiotracer studies (Werner and Hollibaugh 1993).

The relationship between the size of bacterial particles and the efficiency with which they were captured was determined by feeding zebra mussels bacteria of different sizes and measuring the rate at which they were removed from the water (clearance rate). Three strains of cultured bacteria and natural bacterial populations from the Hudson River were utilized (Fig. 3). Over an approximate size range of 0.01–1 μm^3, this relationship could be modeled by a simple first-order regression curve ($r^2 = 0.995$), and the clearance rate (CR) could be calculated as CR = [(1.67 × cell volume) + 0.021] where clearance rate is in units of millilitres per zebra mussel per minute and particle volume cubic micrometres. Highest clearance rates were observed with the largest bacteria, *B. subtilis* (12.7 ± 5.6 μm^3), which were cleared at a rate of 1.3 ± 0.2 mL·zebra mussel^{-1}·min^{-1}. Slightly lower clearance rates were observed for the pseudomonad isolate (strain WJT-1C) used in the feeding experiments (size = 0.54 ± 0.2 μm^3, CR = 0.93 ± 0.1 mL·zebra mussel^{-1}·min^{-1}). Still lower rates were observed for *E. coli* (size = 0.26 ± 0.1 μm^3, CR = 0.44 ± 0.07 mL·zebra mussel^{-1}·min^{-1}) and indigenous river bacteria (size = 0.03 ± 0.04 μm^3, CR = 0.08 ± 0.02 mL·zebra mussel^{-1}·min^{-1}). Thus, unlike filtration of larger sized phytoplankton particles, where clearance rate is relatively unaffected by size (Horgan and Mills 1997), clearance of bacteria-sized particles is highly dependent on size. Filtering efficiency (clearance rate) for large bacteria such as *Bacillus* (1.3 ± 0.2 mL·zebra mussel^{-1}·min^{-1}) is nearly equivalent to reported clearance rates for larger phytoplankton (10–150 μm, 1–3.6 mL·zebra mussel^{-1}·min^{-1}; Horgan and Mills 1997). However, for smaller sized bacteria, representative of native populations, clearance rates are substantially (16-fold) lower. Alternatively, because different species of bacteria were used in these studies, it is possible that some of the differences in clearance rates observed for the different-sized bacterial species may be due to selective grazing. However, since similar results have been reported from filtration depletion studies utilizing different-sized inert microspheres, it seems more plausible that particle size is the primary factor in determining whether cells can be captured by zebra mussels (Lei et al. 1996). Therefore, the size composition of bacterial populations, including the fraction of aggregated bacteria/detritus material, must be considered when estimating the role of bacteria and detritus as a food resource for zebra mussel consumption.

Using the assimilation efficiency of 22.5% determined

Fig. 3. Relationship between bacteria size (volume) and clearance rate by zebra mussel filtration. Bacteria included *B. subtilis* (12.7 ± 5.6 μm^3), *Pseudomonas* sp. (0.54 ± 0.2 μm^3), *E. coli* (0.26 ± 0.1 μm^3), and indigenous river bacteria (0.02 ± 0.04 μm^3). Error bars represent 1 SD of the mean.

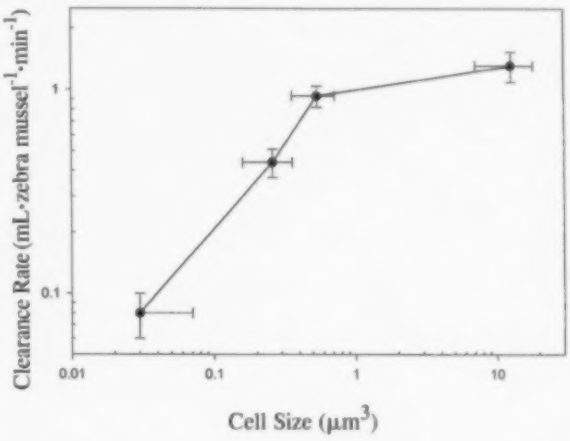

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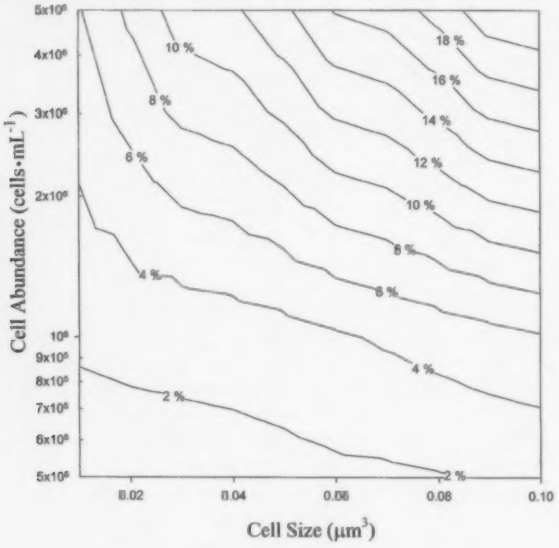
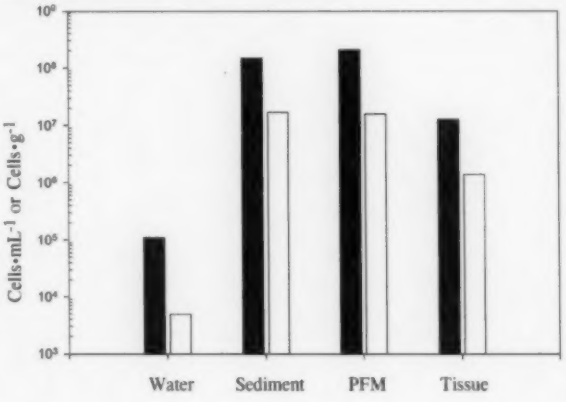
Fig. 4. Model relationship between bacterial abundance, size (volume), and zebra mussel derived growth fraction. The fraction of zebra mussel growth derived from bacteria plankton was calculated as the weight of bacteria assimilated/growth of bacteria expressed as a percentage of the estimated total annual zebra mussel growth (0.1 g dry weight·year^{-1}; M.E. Frischer, unpublished). The weight of bacteria assimilated is calculated as clearance rate × cell abundance × average weight of bacterial cell (3.4×10^{-14} g·cell^{-1}; Fagerbakke et al. 1996) × assimilation efficiency derived in this study (0.225). Clearance rate is estimated as CR = [(1.67 × cell volume) + 0.021], also as determined in this study. Contour lines provide estimates of the fraction of zebra mussel growth attributable to the direct consumption of bacterioplankton.



from experiments where realistic bacteria feeding concentrations (10^6 cells·mL^{-1} at feeding) were used to simulate natural bacterial concentrations and a clearance rate for indigenous aquatic bacteria, also determined in these studies (115 mL·day^{-1}), it is possible to estimate the total amount of zebra mussel growth in the environment that can be directly attributed to the consumption of bacterioplankton. These calculations also assume that indigenous river bacteria, once captured, are nutritionally equivalent by weight to the cultured bacteria used in laboratory feeding studies. For example, in the Hudson River, assuming a bacterial abundance of 10^9 cells·L^{-1}, an average weight of an indigenous bacterial cell of 3.4×10^{-14} g (Fagerbakke et al. 1996), a zebra mussel population size of 550 billion animals (Strayer et al. 1996), and a 280-day growing season, the total annual growth (dry weight) can be calculated as approximately 1.2×10^6 t·year^{-1}. Assuming an average dry weight of 0.1 g·zebra mussel^{-1} and a 3-year life span, the annual growth of zebra mussels in the Hudson River can be estimated to be 5.5×10^7 t·year^{-1}. Thus, by these estimates, growth of zebra mussel populations derived from incorporation of bacterioplankton biomass represents approximately 2% of the total estimated annual growth of zebra mussel populations in the Hudson River. This approach can be utilized to derive a generalized

Fig. 5. Total bacteria (solid bars) and metabolically active bacteria (open bars) associated with water (cells·mL^{-1}), sediments (cells·g^{-1}), PFM (cells·mL^{-1}), and zebra mussel tissue extract (cells·g^{-1}) samples from the Hudson River.



model of the relationship between zebra mussel growth and the consumption of bacterioplankton (Fig. 4). In this model, bacteria-derived zebra mussel growth is estimated as a function of the abundance and size structure of microbial populations using the information empirically derived from this study. Thus, in the Hudson River where bacteria abundance is approximately 10^5 cells·mL^{-1} (Fig. 5) and the average cell size is 0.02 μm^3 (Fig. 3), the amount of zebra mussel growth attributable to the consumption of bacterioplankton is slightly less than 2% (Fig. 4). However, if bacterial abundance is greater, or the bacterial communities are composed of larger cells, a larger fraction of zebra mussel growth may be attributable to the consumption of bacteria. For example, Cotner et al. (1995) reported that in the summer in a eutrophic site in the inner portion of Saginaw Bay (Lake Huron), bacteria could account for 20% of zebra mussel respiratory demand. In this site, bacterial abundance was approximately 1.5×10^6 cells·mL^{-1} and contained bacteria significantly larger than those found in the Hudson River (>0.9 μm in diameter). In the Hudson River, Strayer et al. (1996) estimated carbon assimilation of the zebra mussel populations to be 120–150 g C·m^{-3}·year^{-1} and that primary production was only 50 g C·m^{-3}·year^{-1}. Thus, although no specific measurements were made, these authors speculated that bacteria and detritus must contribute significantly (as much as 67%) to the diet of zebra mussels in the Hudson River. The studies reported here suggest that, at least in the Hudson River, the incorporation of free-living bacterioplankton cannot account for the deficiencies between available phytoplankton production and zebra mussel growth. Thus, future studies should concentrate on the utilization of detritus by zebra mussels.

Impact on microbial communities

Total direct (DAPI) and metabolically active (universal 16S rRNA targeted oligonucleotide probe positive) bacterial counts associated with water, sediments, PFM, and zebra mussel tissue are shown in Fig. 5. Total bacteria counts ranged from 1.1×10^5 cells·mL^{-1} in the water to 2.1×10^8 cells·mL^{-1} in the PFM and the fraction of metabolically ac-

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Fig. 6. Composition of microbial communities associated with zebra mussel environments as defined by phylogenetic group specific 16S rRNA targeted oligonucleotide probes. (A) Sediment and (B) water column environments exhibited the highest diversity of organisms, while (C) PFM and fecal material and (D) zebra mussel tissue homogenates were largely dominated by Gamma and Beta group proteobacteria. In all samples, with the exception of zebra mussel tissue homogenates, a large proportion (24–31%) of the total metabolically active bacteria were not identified by the probes used.

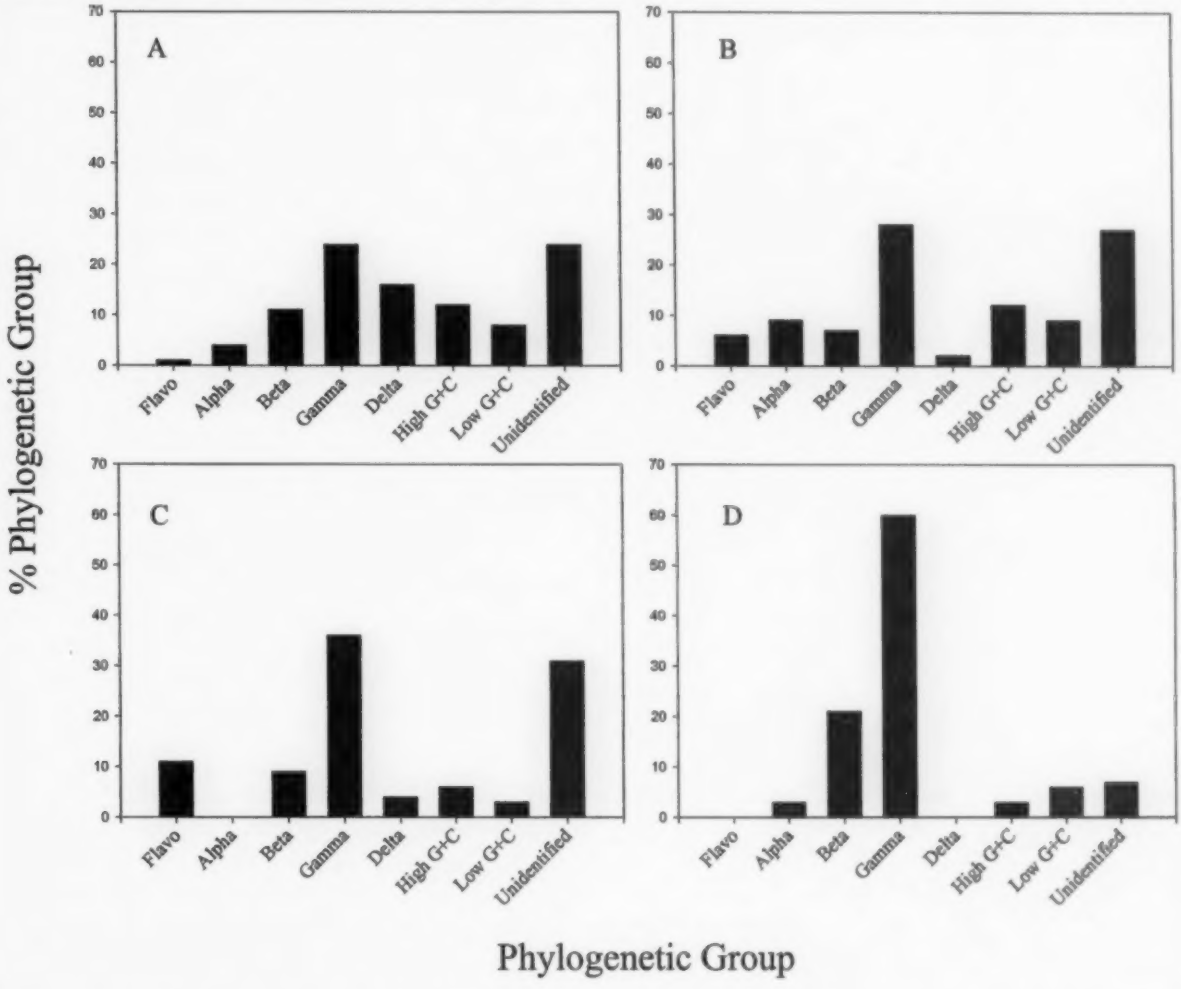

Table 1. Microbial community species diversity and equitability of zebra mussel associated Hudson River samples.

	Water[a]	Sediment[a]	PFM[b]	Zebra mussel tissue[c]
H	2.643	2.672	2.300	1.728
H_{max}	3.00	3.00	2.807	2.585
E	0.881	0.891	0.819	0.668

Note: H is observed species diversity, H_{max} is maximum species diversity, and E is equitability (range = 0–1) calculated using the Shannon–Wiener function (Krebs 1994). Diversity index significantly different ($p < 0.01$) for samples with a different superscript.

tive bacteria ranged from 4.5 to 11% of total bacteria (Fig. 5). Comparison of microbial communities associated with zebra mussels and associated environments (sediments, PFM, and water) using phylogenetic group specific 16S rRNA targeted oligonucleotide probes revealed several interesting differences (Fig. 6). The diversity of microbial communities, as calculated by the Shannon–Wiener function (Krebs 1994), was greatest in the sediments ($H = 2.672$, $E = 0.891$), slightly lower in the water samples ($H = 2.643$, $E = 0.881$), significantly lower in PFM ($H = 2.30$, $E = 0.819$; $p < 0.01$), and lowest in zebra mussel tissue homogenates ($H = 1.728$, $E = 0.668$; $p < 0.01$) (Table 1). In addition to the lowest microbial diversity, the evenness value (E) was also the lowest in tissue homogenates, reflecting the dominance of the Gamma and Beta proteobacteria in these samples. In general, zebra mussel tissue extracts and PFM were enriched in Gamma proteobacteria relative to the water and sediment. Gamma proteobacteria, which include *Vibrios* and relatives of *E. coli*, represented 36 and 60% of the microbial community in PFM and in zebra mussel extracts, respectively, but

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only 28 and 24% of the bacteria in water and sediment, respectively. Gram-positive organisms (High G+C and Low G+C subdivisions), which include *Arthrobacter* and *Bacillus*, were less abundant in zebra mussel tissue extracts and PFM relative to the water and sediment, while other bacterial groups were relatively unchanged between sample types. The Alpha proteobacteria were absent from the PFM samples, while the Delta and Flavo groups were absent from zebra mussel tissue homogenates. These results suggest that zebra mussels and PFM support different types of microbial communities in comparison with sediments and water. Although this observation might have been predicted, the consequences of changes in microbial community structure induced by the presence of zebra mussels may be profound. For example, enrichment of enteric bacteria (e.g., *Vibrio*, *Alteromonas*, *Aeromonas*, *E. coli*, and *Salmonella*) by zebra mussels may impact water quality and public health issues. However, continued investigations will be required to confirm these findings in additional samples and environments, to determine the mechanisms responsible for changing microbial community structure, and to establish cause and effect linkages between alteration in ecosystem functioning and changes in microbial community structure induced by zebra mussels.

Combined, the results of these studies indicate that zebra mussels and bacteria interact directly and indirectly. Feeding studies demonstrated that zebra mussels could utilize bacteria directly as a food source. Consideration of the assimilation efficiency of indigenous bacterial populations suggests that while bacteria can be utilized as a food source for zebra mussel populations, it is unlikely that the direct utilization of free-living bacteria can account for a large portion of zebra mussel growth. In the Hudson River, Strayer et al. (1996) suggested that phytoplankton production was not sufficient to support the large zebra mussel population there. In fact, based on yearly primary production rates of 50 g $C \cdot m^{-3} \cdot year^{-1}$ and an estimated carbon assimilation rate by zebra mussels of 120–150 g $C \cdot m^{-3} \cdot year^{-1}$, it was speculated that two thirds of this carbon may be due to assimilation of bacteria and detritus. Because the findings from this study indicate that only 2% of the annual zebra mussel growth in the Hudson River can be accounted for by the assimilation of free bacteria, it seems likely that detritus or sediment bacterial production, rather then free bacteria, may be a significant food source for zebra mussel populations in the Hudson River.

Our analyses also revealed that microbial communities associated with zebra mussels are quite different from those associated with water and sediments alone and that zebra mussels are likely to promote the enrichment of particular groups of microorganisms. For example, note the enrichment of Gamma and Beta subdivision proteobacteria and the absence of Delta proteobacteria and the Flavo group in zebra mussel tissues relative to sediments and other environments (Fig. 6). Because only a small fraction of the indigenous bacteria population is directly ingested by zebra mussels, it can be concluded that the mechanism by which zebra mussels alter the composition of microbial communities involves the stimulation of specific populations possibly by providing specialized growth substrates or by creating new microniches. While the overall ecological effect and importance of bacteria – zebra mussel interactions remain unclear, the studies presented in this paper indicate that such interactions occur and should therefore be included in future efforts to understand the ecological impact of zebra mussels on aquatic systems.

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References

Barnes, H., and Blackstock, J. 1973. Estimation of lipids in marine animals and tissues: detailed investigation of the sulphosphovanillin method for "total" lipids. J. Exp. Mar. Biol. Ecol. **12**: 103–118.

Braun-Howland, E.B., Danielsen, S.A., and Nierzwicki-Bauer, S.A. 1992. Development of a rapid method for detecting bacterial cells in situ using 16S rRNA-directed probes for identification of common environmental isolates. BioTechniques, **13**: 928–933.

Bruner, K.A., Fisher, S.W., and Landrum, P.F. 1994. The role of the zebra mussel, *Dreissena polymorpha*, in contaminant cycling: I. The effect of body size and lipid content on the bioconcentration of PCBs and PAHs. J. Gt. Lakes Res. **20**: 725–734.

Caraco, N.F., Cole, J.J., Raymond, P.A., Strayer, D.L., Pace, M.L., Findlay, S.E.G., and Fischer, D.T. 1997. Zebra mussel invasion in a large, turbid river: phytoplankton response to increased grazing. Ecology, **78**: 588–602.

Carlton, J.T. 1993. Dispersal mechanisms of the zebra mussel (*Dreissena polymorpha*). *In* Zebra mussels: biology, impacts, and control. *Edited by* T.F. Nalepa and D.W. Schloesser. Lewis Publishers, Boca Raton, Fla. pp. 677–697.

Cotner, J.B., Gardner, W.S., Johnson, J.R., Sada, R.H., Cavaletto, J.F., and Heath, R.T. 1995. Effects of zebra mussels (*Dreissena polymorpha*) on bacterioplankton: evidence for both size-selective consumption and growth stimulation. J. Gt. Lakes Res. **21**: 517–528.

Coughlan, J. 1969. The estimation of filtering rate from the clearance of suspensions. Mar. Biol. (Berl.), **2**: 356–358.

Dietz, T.H., Lessard, D., Silverman, H., and Lynn, J.W. 1994. Osmoregulation in *Dreissena polymorpha*: the importance of Na, Cl, K, and particularly Mg. Biol. Bull. **187**: 76–83.

Fagerbakke, K.M., Heldal, M., and Norland, S. 1996. Content of carbon, nitrogen, oxygen, sulfur and phosphorus in native aquatic and cultured bacteria. Aquat. Microb. Ecol. **10**: 15–27.

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Findlay, S.E., Pace, M.L., and Fischer, D.T. 1998. Response of hetertrophic planktonic bacteria to the zebra mussel invasion of the tidal freshwater Hudson River. Microb. Ecol. **36**: 131–140.

Frischer, M.E., Williams, H.G., Bennison, B., Drake, G.R., Balkwill, D.L., and Paul, J.H. 1996*a*. The naturally transformable marine bacterium "*Vibrio*" WJT-1C is a pseudomonad. Curr. Microbiol. **33**: 287–291.

Frischer, M.E., Floriani, P.J., and Nierzwicki-Bauer, S.A. 1996*b*. Differential sensitivity of 16S rRNA targeted oligonucleotide probes used for fluorescence in situ hybridization is a result of ribosomal higher order structure. Can. J. Microbiol. **42**: 1061–1071.

Holland, R.E. 1993. Changes in planktonic diatoms and water transparency in Hatcher Bay, Bass Island area, western Lake Erie since the establishment of the zebra mussel. J. Gt. Lakes Res. **19**: 717–624.

Holland, R.E., Johengen, T.H., and Beeton, A.M. 1995. Trends in nutrient concentrations in Hatchery Bay, western Lake Erie, before and after *Dreissena polymorpha*. Can. J. Fish. Aquat. Sci. **52**: 1202–1209.

Horgan, M.J., and Mills, E.L. 1997. Clearance rates and filtering activity of zebra mussel (*Dreissena polymorpha*): implications for freshwater lakes. Can. J. Fish. Aquat. Sci. **54**: 249–255.

Karatayev, A.Y., Burlakova, L.E., and Padilla, D.K. 1997. The effects of *Dreissena polymorpha* (Pallas) invasion on aquatic communities in eastern Europe. J. Shellfish Res. **16**: 187–203.

Kepner, R.L., Jr., and Pratt, J.R. 1994. Use of fluorochromes for direct enumeration of total bacteria in environmental samples: past and present. Microbiol. Rev. **58**: 603–615.

Krebs, C.J. 1994. Ecology: the experimental analysis of distribution and abundance. 4th ed. Harper Collins College Publishers, New York.

Lane, D.J., Pace, B., Olsen, G.J., Stahl, D.A., Sogin, M.L., and Pace, N.R. 1985. Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. Proc. Natl. Acad. Sci. U.S.A. **82**: 6955–6959.

Leach, J.H. 1993. Impacts of the zebra mussel (*Dreissena polymorpha*) on water quality and fish spawning reefs in western Lake Erie. *In* Zebra mussels: biology, impacts, and control. *Edited by* T.F. Nalepa and D.W. Schloesser. Lewis Publishers, Boca Raton, Fla. pp. 381–397.

Lei, J., Payne, B.S., and Wang, S.Y. 1996. Filtration dynamics of the zebra mussel, *Dreissena polymorpha*. Can. J. Fish. Aquat. Sci. **53**: 29–37.

Ludyanskiy, M.L., McDonald, D., and MacNeill, D. 1993. Impact of the zebra mussel, a bivalve invader. BioScience, **43**: 533–544.

MacIsaac, H.J., Sprules, W.G., Johannsson, O.E., and Leach, J.H. 1992. Filtering impacts of larval and sessile zebra mussels (*Dreissena polymorpha*) in western Lake Erie. Oecologia (Berl.), **92**: 30–39.

Nicholls, K.H., and Hopkins, G.J. 1993. Recent changes in Lake Erie (north shore) phytoplankton: cumulative impacts of phosphorus loading reductions and the zebra mussel introduction. J. Gt. Lakes Res. **19**: 637–647.

Regan, P.M., Margolin, A.G., and Watkins, W.D. 1993. Evaluation of microbial indicators for the determination of the sanitary quality and safety of shellfish. J. Shellfish Res. **12**: 95–100.

Roberts, L. 1990. Zebra mussel invasion threatens U.S. waters. Science (Washington, D.C.), **249**: 1370–1372.

Roditi, H.A., Caraco, N.F., Cole, J.J., and Strayer, D.L. 1996. Filtration of Hudson River water by the zebra mussel (*Dreissena polymorpha*). Estuaries, **19**: 824–832.

Silverman, H., Acheberger, E.C., Lynn, J.W., and Dietz, T.H. 1995. Latero-frontal cirri in the gill of the zebra mussel, *Dreissena polymorpha*, *Corbicula fluminea*, and *Carunculina texasensis*. Biol. Bull. **189**: 308–319.

Strayer, D.L., Powell, J., Ambrose, P., Smith, L.C., Pace, M.L., and Fischer, D.T. 1996. Arrival, spread, and early dynamics of a zebra mussel (*Dreissena polymorpha*) population in the Hudson River estuary. Can. J. Fish. Aquat. Sci. **53**: 1143–1149.

Strayer, D.L., Caraco, N.F., Cole, J.J., Findlay, S., and Pace, M.L. 1999. Transformation of freshwater ecosystems by bivalves. BioScience, **49**: 19–27.

Verity, P.G., and Sieracki, M.E. 1993. Use of color image analysis and epifluorescence microscopy to measure plankton biomass. *In* Handbook of methods in aquatic microbial ecology. *Edited by* P.F. Kemp, B.F. Sherr, E.B. Sherr, and J.J. Cole. Lewis Publishers, London, U.K. pp. 327–338.

Vescio, P.A., and Nierzwicki-Bauer, S.A. 1993. Extraction and purification of PCR amplifiable DNA from lacustrine subsurface sediments. J. Microbiol. Methods, **21**: 225–233.

Werner, I., and Hollibaugh, J.T. 1993. *Potamocorbula amurensis*: comparison of clearance rates and assimilation efficiencies for phytoplankton and bacterioplankton. Limnol. Oceanogr. **38**: 949–964.

Williams, S.C., Hong, Y., Danavall, D.C.A., Howard-Jones, M.H., Gibson, D., Frischer, M.E., and Verity, P.G. 1998. Distinguishing between living and nonliving bacteria: evaluation of the vital stain propidium iodide and the combined use with molecular probes in aquatic samples. J. Microbiol. Methods, **32**: 225–236.

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Influence of ultraviolet-B radiation, stratospheric ozone variability, and thermal stratification on the phytoplankton biomass dynamics in a mesohumic lake

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Abstract: Terrestrial ultraviolet radiation (UVR) is highly variable in both space and time, and phytoplankton in the mixed layer may be exposed at irregular intervals to significant daily doses. The influence of the natural UVR on phytoplankton dynamics was investigated in a small mesohumic lake, Lac Cromwell, in the Laurentian Hills by means of a time-intensive (about 60 days) daily study of the relationship between UVR flux and phytoplankton biomass. Following the onset of lake stratification, at which time the epilimnion became shallower than 2.5 m, the study revealed a strong negative relationship between ultraviolet-B radiation (UVB) and algal biomass at the surface (r^2 = 0.61) and at 1 m (r^2 = 0.38). Although this relationship held throughout the stratified period, chlorophyll *a* concentration declined particularly rapidly (–65%) during a short-lived ozone-thinning period. There was a major shift in the community taxonomic composition during the same period from a typical diatom–chrysophyte spring bloom towards a dinoflagellate-dominated community that was followed by cyanobacteria. Here, we present evidence that the impact of the temporary increase in UVB was intensified by a concurrent lack of mixing, indicating that turbulence and thermal stratification are key components modulating UVB effects in lakes.

Résumé : Le rayonnement ultraviolet (UV) terrestre varie beaucoup selon le lieu et dans le temps, et le phytoplancton de la couche de mélange peut être exposé à des doses journalières importantes, à des intervalles irréguliers. On a examiné l'influence des rayons UV naturels sur la dynamique du phytoplancton du lac Cromwell, un petit lac mésohumique des Laurentides, en procédant à l'étude intensive (environ 60 jours) de la relation entre le flux des rayons UV et la biomasse de phytoplancton. Après que le lac ait commencé à se stratifier, et alors que l'épilimnion atteignait une profondeur de moins de 2,5 m, l'étude a révélé une forte relation négative entre les rayons ultraviolet-B (UVB) et la biomasse algale à la surface (r^2 = 0,61) et à 1 m (r^2 = 0,38). Quoique cette relation se soit maintenue pendant toute la période où le lac était stratifié, la concentration de chlorophylle *a* a chuté particulièrement vite (–65%) pendant une courte période d'appauvrissement de la couche d'ozone. Des modifications importantes de la composition taxonomique du phytoplancton ont été observées pendant cette période : après la prolifération printanière typique de chrysophytes et des diatomées, il y a eu dominance par les dinoflagellés, suivie par l'apparition de cyanobactéries. Nous montrons que l'impact de la hausse temporaire du rayonnement UVB a été amplifié par l'absence concomitante de mélange, ce qui indique que la turbulence et la stratification thermique sont des facteurs qui modulent de façon importante l'effet des rayons UVB dans les lacs.

[Traduit par la Rédaction]

Introduction

Solar ultraviolet-B radiation (UVB), whether at current or increased levels, represents a stress on aquatic ecosystems. Ambient UVB can negatively affect organisms at all trophic levels both directly via physiological damage (Karentz et al. 1994) and indirectly via food chain effects or development of toxic photoproducts (Bothwell et al. 1994; Xenopoulos and Bird 1997). Removing natural UVB can radically alter the taxonomic composition of marine phytoplankton communities, thereby changing the dynamics of trophic interactions and carbon flux (Davidson et al. 1996). Although exposure to UVB may stimulate microbial production by releasing growth-limiting nutrients in small-scale experiments (e.g., Laurion et al. 1998), less is known about the net effect of UVB at the ecosystem scale.

In evaluating the impact of UVB on marine and freshwater phytoplankton, it has been difficult to distinguish the individual significance of the many environmental variables that directly control growth rate, such as temperature, nutrient availability, ultraviolet radiation (UVR), and photosynthetically active radiation (PAR), all of which tend to covary over the growing season or from one day to the next. It is not clear where UVR stands in the hierarchy of these influences or how observed responses to changes in UVR are

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influenced by other environmental factors. An ecosystem that has been deprived of nutrients or subjected to unusual extremes of temperature might respond to a change in UVR differently than would a system that is not otherwise under stress.

In theory, the impact of UVB on pelagic freshwater organisms depends on the rate of vertical mixing as well as on the mixing depth (Neale et al. 1998). Unfortunately, the vertical movement of planktonic organisms in the epilimnion is difficult to model or imitate in experiments, so there is a paucity of information on the true UVB impact in the natural environment. Normally, an epilimnion is present in temperate lakes during the summer, ranging in depth from a few metres in small lakes to more than 10 m in very large lakes. The exposure to damaging UVR in this water stratum varies according to the vertical movement of the plankton and the time spent in UVR-exposed upper levels in the water column. A further impediment to theoretical estimation of natural UVR impact is the possibility that the radiation dose, defined as irradiance (watts per square metre) times exposure time, might engender a damage response that does not follow a simple photochemical reciprocity law (Cullen and Lesser 1991). In other words, short intense exposures might be more or less damaging than low but extended exposures.

This study attempted to determine the longer term community-level response to UVB in a natural aquatic environment. The objective was to establish empirical relationships between UVB, PAR, temperature, and phytoplankton biomass on a day-to-day scale by performing an intensive time series study in a small mesohumic lake. We sought to separate the influence of UVR, PAR, and temperature and thereby to establish their importance for phytoplankton dynamics.

Materials and methods

Lake characteristics

Lac Cromwell (45°30′N, 74°30′W) is a small humic lake (8 ha, z_{max} = 9 m, water residence time <1 months) on the Canadian Shield (Quebec, Canada). Mean total phosphorus concentration is 9 $\mu g \cdot L^{-1}$ and pH values fall between 5.5 and 6.5. Dissolved organic carbon (DOC) concentration ranges from 7 to 10 $mg \cdot L^{-1}$. After ice breakup, stratification is established quickly, often before the hypolimnion can be completely aerated. The hypolimnion and even the metalimnion are usually anoxic from top to bottom by midsummer. The limit of the euphotic zone (1% light level), which is determined largely by DOC but also by chlorophyll, is 2–3 m. The "true" euphotic depth is much deeper; a large community of photosynthetic bacteria (largely *Chlorobium limicola*) develops at 6 m in midsummer. UVR attenuation depths (1%) as estimated based on equations derived in this lake by Scully and Lean (1994) are 0.56 m for ultraviolet-A radiation (UVA) and 0.21 m for UVB.

Sampling protocol

Vertical profiles of chlorophyll *a*, oxygen, and temperature were measured once daily between 9 and 10 a.m. beginning at ice-out (5 May 1994) and finishing midsummer (8 July 1994). Water samples were collected from the surface (first 25 cm) and at 1, 2, and 3 m depths at the deepest station of the lake. Water was collected with a Van-Dorn bottle during May and with a peristaltic pump (Masterflex) during June and July.

Chlorophyll *a* and floristic analyses

Water for chlorophyll measurement was collected in dark Nalgene bottles and filtered on a GF/F filter. Chlorophyll was immediately extracted, in duplicate, with boiling ethanol (95%), as described in Sartory and Grobbelaar (1984), and kept cold until spectrophotometric reading.

For cell counts and specific composition, duplicate 20-mL samples were collected every 2 days from the surface and at 1 m, fixed with Lugol's solution and formaldehyde, and kept at 4°C until counted. Samples (10 mL) were left to settle for 24–48 h and inverted microscope observations of phytoplankton at 400× magnification were used to record species composition and cell numbers.

Solar radiation and ozone concentration

Solar UVB and ozone concentration data were provided by Environment Canada through the World Ozone and Ultraviolet Radiation Data Centre (WOUDC) (website: http://www.Tor.ec.gc.ca/woudc). UVB was measured with a Brewer spectrophotometer at Dorval (Quebec, Canada), approximately 80 km south of Lac Cromwell, at regular intervals throughout the day. The spectral irradiances were then weighted according to different action spectra and integrated over the day. Details of instrumentation and precision of UVR measurements and ozone concentration are presented in Kerr and McElroy (1993).

Total solar radiation was also provided by Environment Canada. It was converted to PAR by multiplying by 0.45 (Kirk 1994). PAR is essentially unaffected by changes in stratospheric ozone thickness.

Four different indices of solar damage, produced by daily integration of four biological weighting functions, were tested for their ability to explain phytoplankton dynamics. These were the CIE standard erythemal action spectrum (CIE) (McKinlay and Diffey 1987), a spectral biological weighting function for the inhibition of photosynthesis, by UVR, for a laboratory culture of the marine temperate diatom *Phaeodactylum* sp. (PS) (Cullen et al. 1992), the DNA-damage action spectrum (UV_{DNA}) (Setlow 1974), and the ACGIH-NIOSH spectrum, a general measure of biological damage that was developed using laboratory data from many species to establish guidelines for occupational exposure (ACGIH) (ACGIH-NIOSH 1972). Both CIE and ACGIH were taken from the WOUDC. Above 300 nm, the importance accorded to UVB (290–315 nm) relative to UVA (315–400 nm) increases roughly in the order PS < CIE < ACGIH < UVB_{DNA}.

Temperature and oxygen

Vertical temperature profiles of the lake were measured daily at the time of sampling. Temperature was also measured continuously at the surface and at 0.5, 1.5, and 3 m depths from 28 May 1994 until 24 June 1994 at 10-min intervals with Hobo-Temp in situ data loggers. Both temperature determinations were used to estimate daily mixed layer depth. Oxygen determinations at every metre were made by Winkler titration.

Statistical analysis

Correlations, indicated by the correlation coefficient r, were used to determine the strength of the relationships between biological weighting functions, temperature, chlorophyll, ozone, and PAR. When chlorophyll dynamics were modelled, 3-day moving averages of chlorophyll *a*, weighted UVB, PAR, and temperature were used in ordinary least squares regression analysis to integrate the light effects over time and to reduce chlorophyll sampling error (shown using r^2). Otherwise, daily observations were used. All reported coefficients were significant at the 5% probability level; p values are shown when relationships were not significant.

Results

Biological weighting functions

The four solar damage indices (CIE, PS, UVB_{DNA}, and ACGIH) were strongly correlated with each other. The low-

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est correlation ($r = 0.90$) was between the indices at the two extremes, PS (most strongly affected by UVA) and UVB_{DNA} (most strongly weighted towards UVB). The relationship between the two was significantly nonlinear, varying according to ozone thickness and time of the year. Preliminary analysis showed that chlorophyll concentration at the surface of the lake was most strongly correlated with UVB_{DNA} ($r = 0.63$) and less strongly with PS ($r = 0.49$). The following description of UVB effects is therefore based on the UVB_{DNA} index.

Solar radiation, ozone concentration, and temperature

Strong thermal stratification was established in the lake by 20 May 1994. The depth of the epilimnion was generally shallower than 2.5 m after this date (mean 1.5 m, CV 75%). Mean daily temperature at the surface increased from 4°C during ice-break to 26°C in mid-June. Mean temperature at the surface of the lake after induction of stratification was correlated with UVB_{DNA} ($r = 0.45$, $n = 49$).

During the two spring and early summer months of this study, levels of stratospheric ozone varied remarkably through time, by up to 26%, ranging between 295.3 and 411.5 Dobson units. Ozone concentration was negatively correlated with UVB_{DNA} ($r = -0.55$, $n = 49$) but was not correlated with PAR ($r = -0.20$, $p = 0.17$, $n = 49$). The correlation between PAR and solar UVR, weighted for DNA damage (UVB_{DNA}), was strong ($r = 0.87$, $n = 49$) but stronger for UVR weighted for photoinhibition ($r = 0.96$, $n = 49$). The correlation between UVB_{DNA} and ozone concentration is weak because the intensity of UVB radiation at the earth's surface is not only a function of the thickness of the ozone layer but also depends on the solar angle, atmospheric aerosols and pollutants, and cloud cover (Madronich et al. 1995). The best model to predict UVB_{DNA} depended on both PAR (megajoules per square metre) and ozone thickness (Dobson units): $UVB_{DNA} = 1312 + 69.4(PAR) - 3.43(O_3)$ ($r^2 = 0.89$).

Mixed-layer phytoplankton chlorophyll *a*

Between ice-out and the establishment of stratification, there was no relationship between UVB and lake chlorophyll concentration, presumably due to light and temperature limitation of growth. However, from 20 May 1994 onwards, we found a strong negative relationship between UVB_{DNA} (joules per square metre) and phytoplankton biomass as measured by chlorophyll content (micrograms per litre) at the surface (Fig. 1*a*) and at 1 m (Fig. 1*b*):

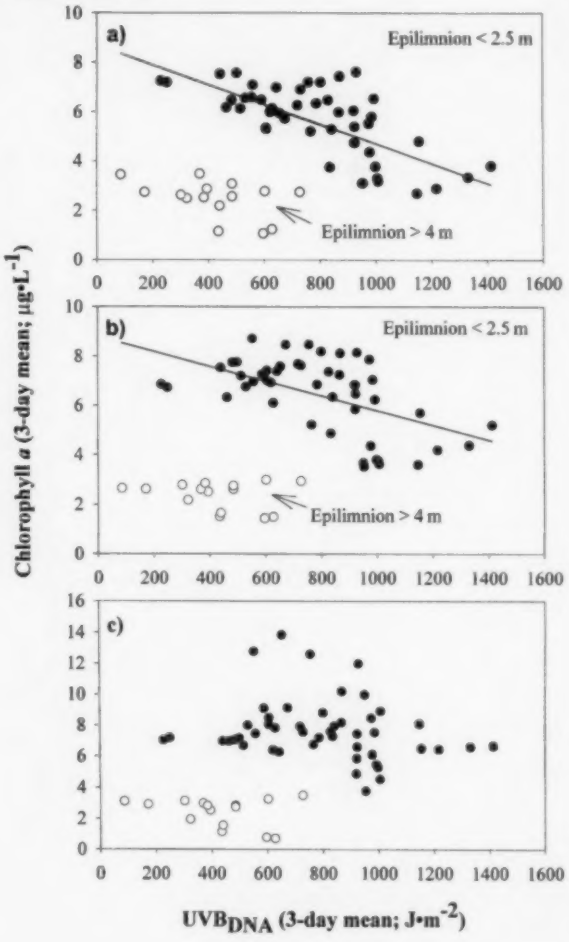
(1) 0 m: $Chl = -0.004(UVB_{DNA}) + 8.7$
($r^2 = 0.61$, $n = 42$, mean Chl = 5.4)

(2) 1 m: $Chl = -0.003(UVB_{DNA}) + 8.8$
($r^2 = 0.38$, $n = 42$, mean Chl = 6.2).

Note that the relationship obtained at 1 m was significantly weaker than it was at the surface of the lake ($p < 0.05$). There was no significant univariate relationship between UVR and chlorophyll at depths >1 m ($r^2 = 0.05$ at 2 m, $p = 0.17$, $n = 42$; $r^2 = 0.01$ at 3 m, $p = 0.48$, $n = 42$) (Fig. 1*c*).

Multiple ordinary least squares regressions relating chlorophyll (micrograms per litre) to UVB_{DNA} (joules per square metre, weighted), ozone (Dobson units), and day of the year (time) also yielded significant models:

Fig. 1. Relationship between chlorophyll *a* concentration and UVB_{DNA} at (*a*) the surface of Lac Cromwell and at (*b*) 1 m and (*c*) 2 m. Regression lines are illustrated in Figs. 1*a* and 1*b*. Open and solid circles separate the two stratification periods (open circles, epilimnion >4 m; solid circles, epilimnion <2.5 m). Solid circles only were used in the regression analysis. After 20 May 1994, the reduction in the depth of the epilimnion restricted the phytoplankton at the surface (0 and 1 m) where they were potentially exposed to more harmful UVB radiation for a longer period. Hence, only the period after 20 May was considered in the regression analysis.



(3) 0 m: $Chl = -0.0028(UVB_{DNA}) + 0.021(O_3) + 0.052(time) - 8.1$ ($r^2 = 0.78$)

(4) 1 m: $Chl = -0.0023(UVB_{DNA}) + 0.019(O_3) + 0.063(time) - 8.8$ ($r^2 = 0.66$).

The impact of UVB was negative whereas ozone thickness had a positive effect at the surface. At 1 m, the effect of UVB was still apparent. The effect of UVB on chlorophyll in the lake always entered first in stepwise regression analyses and was the strongest variable in the models. Tempera-

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ture entered into the models only when time was excluded; its apparent effect at the surface could not be separated from its correlation with time. We believe that the presence of ozone thickness in these models acts as a spectral correction to the Setlow weighting function, a hypothesis that we are now pursuing to derive an in situ action spectrum specific to this lake. The effect of UVB_{DNA} was not a time series artifact; this factor was also significant in simple autoregressive models of chlorophyll concentration in which temporal autocorrelation is removed. The partial correlation (r^2) of UVB_{DNA} with chlorophyll concentration, controlling for the temporal trend, was 69% at 0 m and 49% at 1 m.

Floristic composition

We distinguished two main phytoplankton communities in Lac Cromwell. The typical "spring bloom" community was present until 18 June 1994 and was composed mainly of Chrysophyceae and Bacillariophyceae with some Dinophyceae, Cryptophyceae, and Desmidiaceae (Fig. 2). The second growth period (mid-June until the end of the study) was dominated by blue-green algae (Fig. 2), mainly *Merismopedium*, *Synechococcus*, and *Chroococcus*. This shift in community composition coincided with the period of the highest UVB (between 15 and 20 June 1994).

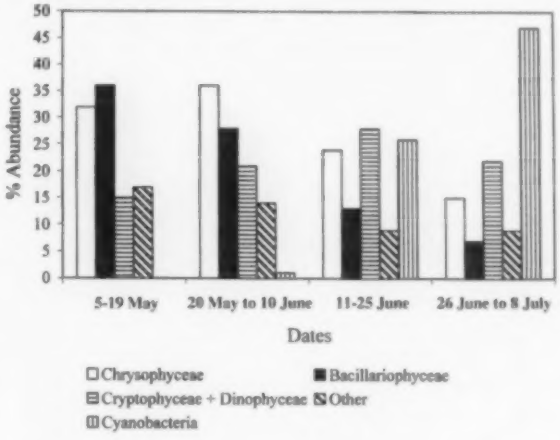
Ozone depletion and mixing

The mixing regime was directly implicated in the negative effects of UVR on phytoplankton dynamics. Between 11 and 20 June 1994, a stratospheric ozone thinning event occurred (Fig. 3). By chance, cloudless skies predominated along with very low wind and an increase in surface temperatures. As a result, a shallower second thermocline developed at <0.5 m on 11 June and remained until 19 June 1994 (Fig. 4). Not surprisingly, ozone and UVB_{DNA} were inversely related during this period (r = –0.73). This event produced three marked effects on the phytoplankton community. First, the ozone thinning matched a drastic decrease of about 65% in chlorophyll *a* (Figs. 3 and 5) at the surface of the lake. The chlorophyll at 2 and 3 m depth increased after a period of 2–3 days (Fig. 5). Second, a shift in the community composition was seen during that same period from a diatom–chrysophyte assemblage typical of humic lakes towards a dinoflagellate-dominated community and then to cyanobacteria (Fig. 2). Third, concentration of dissolved oxygen and percent oxygen saturation, which are measures of the photosynthetic activity of the plankton community relative to respiration, declined markedly during the shallow epilimnion-enhanced UVB event (r = 0.88) (Fig. 6). The decline in percent oxygen saturation occurred despite a decline in bacterial abundance (not shown) and the increase in water temperature (Fig. 4).

The significant interaction of mixing depth and UVB damage in the control of phytoplankton biomass at the surface of the lake throughout the stratified period is demonstrated in the following equation:

$$(5)\quad Chl_{0m} = -0.0036(UVB_{DNA}) + 0.0012(UVB_{DNA} \times \text{mixdepth}) + 7.1 \qquad (r^2 = 0.84,\ n = 41)$$

Fig. 2. Evolution of most abundant taxa during four periods: (*i*) before stratification (5–19 May 1994), (*ii*) stratified period or spring growth period (20 May to 10 June 1994), (*iii*) ozone depletion period (11–25 June 1994), and (*iv*) summer growth period (26 June to 8 July 1994). The taxonomic structure of this lake shifted from a diatom–chrysophyte assemblage towards a dinoflagellate-dominated community followed by dominance by cyanobacteria.



where mixdepth is the thickness of the epilimnion (metres). The interaction term indicates that the greater the mixing depth, the weaker the effect of UVB, and therefore the stronger the phytoplankton community. This relationship held equally well outside the late-June ozone-thinning event. Solution of the equation suggests that no UVB effect on epilimnetic phytoplankton would have been seen at mixed layer depths >3 m in this lake (such as before the mid-May shallow stratification). The 3-m depth was six times the UVA attenuation depth (i.e., 1% of surface UVA) and 14 times the UVB attenuation depth. It is also interesting to note that the addition of a term for the interaction of mixing depth and UVB in eq. 5 provides a more powerful predictive equation explaining more variation than does UVB alone in eq. 1, as shown by the increase in r^2.

Although UVB was not at all directly correlated with chlorophyll concentration at 2 and 3 m depths, statistical models describing the dynamics of phytoplankton biomass below the mixed layer show a significant influence of UVB, mixed layer depth, and temperature on the deep communities through their historical effects on phytoplankton at the surface and thereby on recruitment to deep layers by sedimentation. In the following equations, lag(n) X refers to the value that variable X had displayed n days earlier. Thus, lag(2) Chl_{0m} is the value of chlorophyll concentration at the lake surface 2 days earlier. The 2-m depth was in and out of the epilimnion and was transitional:

$$(6)\quad Chl_{2m} = 2.4(\text{lag}(3)\ Chl_{0m}) - 0.17(\text{lag}(3)\ Chl_{0m})^2 - 0.04(\text{lag}(2)\ O_3) - 0.004(\text{lag}(2)\ UVB_{DNA}) - 0.8(\text{lag}(2)\ \text{mixdepth}) + 18.2 \qquad (r^2 = 0.63,\ n = 39).$$

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Fig. 3. Variations in chlorophyll *a* concentrations at the surface (0 m) of Lac Cromwell over time with fluctuations of ozone concentration. Discontinued lines indicate missing data.

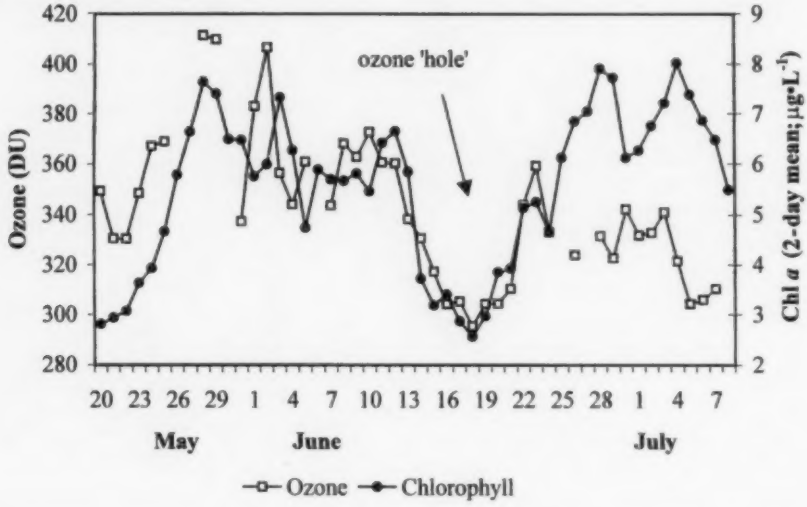


Fig. 4. Average daily temperature measured with in situ data loggers at the surface and at 0.5 and 3 m during the ozone depletion period. A diurnal secondary thermocline was established 11 June 1994 when temperatures rose above 20°C during the day; the epilimnion was then always shallower than 0.5 m until 19 June, 1994.

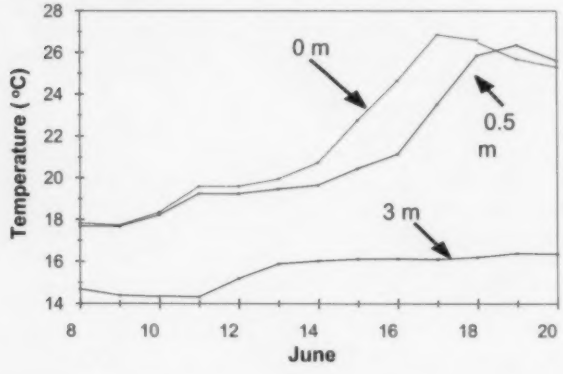
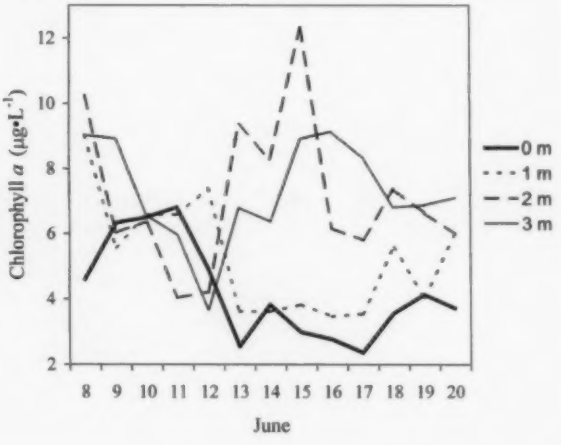


Fig. 5. Variation in chlorophyll concentration at the surface and at 1, 2, and 3 m during the ozone depletion period (11–17 June 1994).



Chlorophyll at 3 m depth was also strongly dependent on events at the surface in the preceding 3 days:

$$\text{Chl}_{3m} = 14.7(\text{lag(3) Chl}_{0m}) - 2.6(\text{lag(3) Chl}_{0m})^2 + 0.14(\text{lag(3) Chl}_{0m})^3 - 0.0016(\text{lag(2) UVB}_{DNA}) + 0.20(\text{lag(2) temp}) - 20.4 \quad (r^2 = 0.78,\ n = 42) \tag{7}$$

where temp was the surface temperature (temperature at 3 m was more or less constant, Fig. 4). The logical interpretation is that chlorophyll levels at 3 m were controlled by levels at the surface 3 days previously, modified strongly by UVB and temperature acting on that chlorophyll 2 days previously, both through their effects on recruitment to deeper layers and through removal of phytoplankton, thereby permitting more light to reach 3 m (Fig. 7).

Discussion

PAR varies gradually between seasons and varies markedly from day to day with changes in cloud cover, but most aquatic ecologists are currently unaware of the common occurrence of large fluctuations in stratospheric ozone at both seasonal and daily scales in northern latitudes. These thinnings change the spectral distribution of the radiation impinging on lakes; the present study suggests that the quality of the light falling on the plankton strongly affects community biomass on the daily scale, and perhaps community composition on longer time scales. Ozone does not effectively absorb the longest wavelengths of UVR (UVA, 320–

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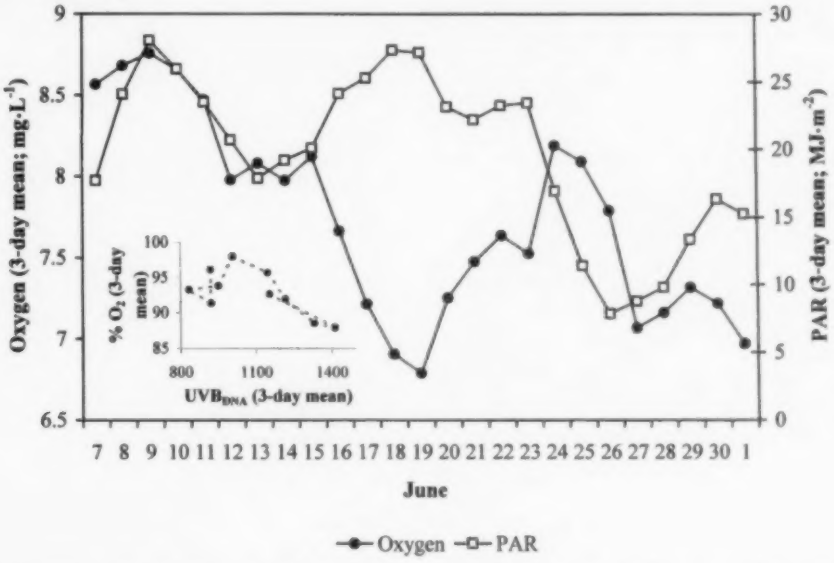
Fig. 6. Variation in surface PAR and dissolved oxygen (0 m) and the relationship between percent oxygen saturation and UVB_{DNA} (inset) during the ozone depletion period.



400 nm), and incident levels of this radiation are largely unaffected by variations in ozone concentrations. As a consequence, ozone loss produces a shift in the ratios UVB:UVA and UVB:PAR and hence a shift between DNA damage and light repair in microorganisms (Jeffrey et al. 1996).

Middle and higher latitudes of the Northern Hemisphere may be particularly likely to experience local ozone depletion (Edouard et al. 1996). During our study, a reduction in the ozone column of approximately 80 Dobson units (30%, Fig. 3) occurred naturally over a few days. The associated increase in damaging UVB could potentially be devastating for surface organisms; in this study, the surface phytoplankton community declined by up to 65%, although other factors were acting at the same time (see below). Nevertheless, the decline seen during the ozone-thinning event was of the size and direction predicted on the basis of dynamics seen throughout the rest of the spring period in response to changes in UVB. The conclusion that can be drawn from these observations is that high-energy UVB wavelengths had a negative impact on phytoplankton health and succession in this lake from the onset of stratification, continuing at least until the establishment of a different midsummer community.

Examination of the stratospheric ozone data over Montreal from the year previous to this study (1993) and the subsequent year (1995) indicated that a decline of >60 Dobson units in a few days (<7 days) is not uncommon and can occur three or four times in a year. Without direct measurement of UVB radiation, plankton ecologists will be unaware of these large week-to-week and even day-to-day variations in incident UVR energy that appear capable of driving community structure and function. The evident possibility for environmental damage associated with declines in stratospheric ozone thickness is cause for concern. However, our results demonstrate that at *current* levels (natural or not), UVR and ozone-thinning can have large effects on lake ecosystems. In fact, the biomass of the plankton as measured by chlorophyll content was more sensitive to UVB fluctuations than it was to variations in the commonly measured PAR on which phytoplankton growth depends.

A major problem in the evaluation of the impact of enhanced UVB radiation on marine and freshwater phytoplankton has been to account for the effect of the vertical mixing regime of the algal cells in the water column, which can in itself mitigate or exacerbate the damages (Cullen and Lesser 1991). The synergy of bright solar irradiance, solar heating of the surface water, and the calm winds that accompany summer high-pressure systems can result in the formation of near-surface thermoclines (Imberger 1985). Under these circumstances, the phytoplankton of clear lakes are retained under photoinhibitory irradiances for prolonged periods of times (Milot-Roy and Vincent 1994). Our study shows that the extent and magnitude of damaging UVB effects can be greatly increased depending on the nature of the stratification regime. Indeed, the effects are compounded: enhanced solar radiation can be damaging to the phytoplankton, especially at higher temperatures with restricted nutrient recycling (Butow et al. 1998), and the accompanying shallow surface thermocline eliminates the possibility of respite and repair in darker waters.

UVB penetration in aquatic systems is a function of the optical properties of the water, especially the amount of dissolved organic matter (Scully and Lean 1994). When water is rich in humic substances and high in colour, planktonic organisms are only exposed to UVR at the uppermost surface levels. In Lac Cromwell, UVR is attenuated in the first decimetres of the water column. It has been suggested that this humic content provides protection for the planktonic community. However, despite the rapid attenuation of UVB in Lac Cromwell and the potential for refuge from UVB in the lower portion of a well-mixed water column, this study suggests that photorepair could not fully compensate for physiological and genetic damage because rapid light attenu-

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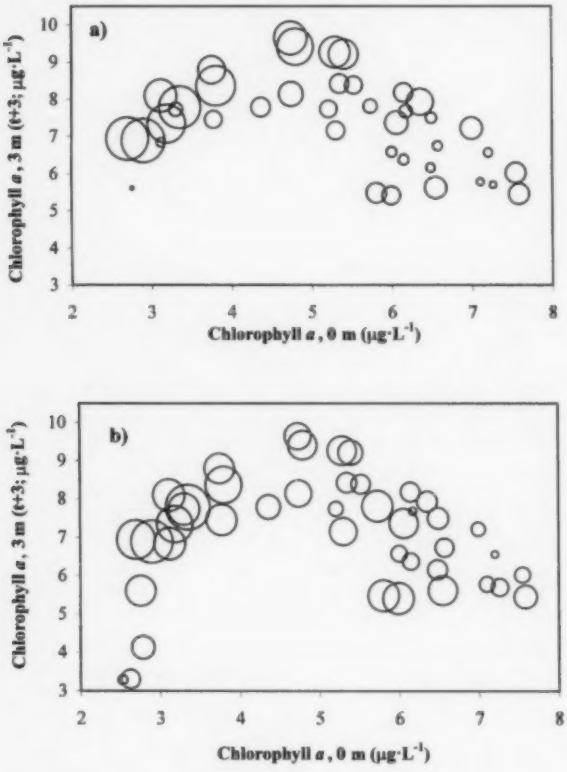
Fig. 7. Bubble plots of the temporal relationship between metalimnetic chlorophyll *a* and surface chlorophyll *a* in Lac Cromwell. Equation 7 shows that surface chlorophyll *a* has a nonlinear relationship with metalimnetic chlorophyll with a time lag of 3 days. (*a*) The radius of the bubbles is proportional to temperature (degrees above 12°C) with a time lag of 1 day. When surface chlorophyll was high, deep chlorophyll was low 3 days later; decreasing surface chlorophyll permitted more growth at 3 m. The largest bubbles are associated with the near-surface thermocline ozone-thinning event in mid-June, when the surface community declined dramatically without recruiting to 3 m depth. (*b*) The radius of the bubbles is proportional to UVB_{DNA}.



ation led naturally to a sharp shallow thermocline. Thus, in addition to the UV-optical depth, mixing depth is a key factor through which UVR damage is moderated (Scully et al. 1998). It seems likely that the probability of surface diurnal thermoclines will be greater in humic than in clear lakes. Two dimensionless numbers could, theoretically, indicate the relative danger to the plankton in any lake. These are the ratio of the absorption coefficients of UVB and PAR, which would vary among lakes, and the ratio of UV-optical depth and mixing depth, which would vary among lakes and among seasons within a lake. The former is increased by coloured DOC, whereas the latter may not vary with DOC, but it may rather be a function of lake latitude and drainage basin morphometry.

But there is a further complication to the interaction of lake colour, stratification, and UVR damage. That is, in addition to direct radiation damage to cellular material, the interaction between light and DOC produces toxic photoproducts (Cooper and Zika 1983; Xenopoulos and Bird 1997). The surface concentration of peroxides and other activated oxygen species will be greater in more humic stratified waters. The role played by these indirect UVR effects here is not known but is expected to be complex. We have shown, for example, that the phytoplankton of this lake are consistently better able to cope with oxidative stress than are the bacteria (Xenopoulos and Bird 1997). The significant interaction between UVB and mixing depth in eq. 5 is in the direction predicted by this indirect damage route. However, we suggest that the greater predictive success of UVB_{DNA} relative to other weighting schemes indicates that direct cellular damages are more important than indirect, since peroxides are not only formed by UVB. It is also possible that the interaction between DOC and UVR may have increased the photochemical oxygen consumption, thus explaining the decrease seen in dissolved oxygen during the ozone depletion period (Fig. 6) (Lindell and Rai 1994).

Avoidance, or migration, can be a biological strategy for minimizing UVR damage (Karentz 1994). However, phytoplankton are largely, although not entirely, at the mercy of physical turbulence; not many species can undertake migrations, especially in water columns with strong vertical gradients of temperature and chemical conditions (Jones 1993), as was the case in this lake. In addition, motility and orientation can be impaired by UVB (Ekelund 1990). Although there is no significant overall relationship between UVB_{DNA} and chlorophyll *a* at 2 m, where UVR was almost completely absent, we did nonetheless distinguish an increase in phytoplankton chlorophyll *a* specifically during the ozone-thinning period (Fig. 5). The taxonomic composition of the phytoplankton was not determined at 2 and 3 m depths, and therefore we cannot say whether a migration or settlement occurred. However, the indirect influence of UVB on chlorophyll dynamics at 2 and 3 m depths was demonstrated clearly in eqs. 6 and 7 through sinking from the surface or recruitment. Among the phytoplankton species of Lac Cromwell, vertical migration would normally be possible for the flagellates, such as *Cryptomonas* and the dinoflagellates, that were the most abundant taxa during the ozone-thinning period. The abrupt increase in chlorophyll at 2 m, however, suggests that the diatoms also sank out of the surface layer of the lake. However, the increase in chlorophyll at 2 m depth was greater than the loss at the surface (0 and 1 m). On the other hand, a close examination of the temporal record showed that the drastic epilimnetic decline was over before the increased light regime permitted a slow increase in chlorophyll seen at 3 m where the phytoplankton are limited by light.

Beyond the direct loss of biomass, we considered the possibility that the negative correlation observed between weighted UVR and chlorophyll concentration was due to direct photooxidation of pigments without other injury to algal cells, or due to simple photoadaptation (Falkowski and LaRoche 1991). Most evidence suggests that destruction of photosynthetic pigments or damage to chloroplasts is the major UVR inhibitory effect. Full sunlight increases the carotenoid to chlorophyll *a* ratio, which may provide photoprotection for the phytoplankton (Paerl et al. 1983);

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change in the ratio was a result of increased carotenoid content per cell. Once again, here, because the DNA damage weighted radiation explained more variation (via higher predictive power) relative to the photoinhinbition spectrum, we believe that the effects seen here went beyond photoinhibition or photoadaptation and that cells were more seriously damaged, lysed, or sank permanently to deeper layers of the lake. For this reason, we believe that these results demonstrate the power of UVR to strongly alter phytoplankton dynamics in this boreal lake.

The coincidence of intensified UVR with the shift seen in species composition in the lake raises the question of the unrecognized role that damaging radiation may play in species succession in aquatic ecosystems. Without necessarily considering anthropogenic declines in ozone levels, we suggest that the ecological role of natural UVB variability in phytoplankton dynamics has been underestimated. Increases in surface UVB resulting from ozone depletion may provide UVB-tolerant species an advantage in nutrient competition, for example. Development of vertical structure and elevated surface temperatures are known to cause a shift in the composition of phytoplankton communities and promote cyanobacterial dominance (McQueen and Lean 1987; Zhang and Prepas 1996). What is the role of increased UVB intensity in this community shift? We hypothesize that in addition to high temperature and PAR, acute UVR stress apparently contributed to the replacement of the chromophytes by the more robust cyanobacteria and dinoflagellates.

Cyanobacteria have a broad range of strategies and repair defenses against UVR. For instance, the UVB-induced production of the cyanobacterial pigment scytonemin has been found to confer protection against UVR damage (Garcia-Pichel and Castenholz 1991). Kana et al. (1988) claimed that the carotenoid zeaxanthin, shown to increase relative to chlorophyll in light-stressed cyanobacterial blooms, is strictly for light protection and plays no role in photosynthesis. Given the capacity to develop protective pigments, and the major asymmetry in ozone depth between spring and fall in the Northern Hemisphere, it is interesting to speculate that UVB may be an additional factor explaining the shift to a cyanobacteria community composition in temperate freshwater ecosystems.

The increase in dinoflagellates following the ozone-thinning event may also be related to relative tolerance to UVB. Some dinoflagellates exhibit positive phototaxis in the presence of humic substances (Nielsen and Ekelund 1993) and gather near the surface during favourable growth conditions (i.e., abundant nutrients), where they may be exposed to high UVR, producing the UV-photoprotective pigment MAA (Carreto et al. 1990).

Is the apparent UVB impact on the phytoplankton in this study unique to Lac Cromwell or are the results applicable to other aquatic systems? Studies of the importance of UVR in other systems have been contradictory (e.g., Hill et al. 1997), possibly because natural communities can acclimate to UVR (either physiologically or by elimination of weaker species), so that vulnerability depends strongly on exposure history. Therefore, the altitude and especially the latitude of the habitat, with the associated local seasonal variations of dose rate, may be a critical determinant of UVR vulnerability: the most highly exposed organisms will also be the most highly protected and adapted. The observation that plankton at tropical latitudes are not as sensitive as those in more temperate latitudes (Helbling at al. 1992) supports this generalization.

We hypothesize that these results will be a consistent feature of the community present until the arrival of the summer solstice when solar radiation reaches its peak. The most vulnerable community is the one present under the ice and present until stratification (diatoms, chrysophytes) because previous exposure is minimal. The risk of UVB damage, and of microstratification, increase rapidly until late June, when a critical UVR event becomes more or less inevitable. Recent evidence from Lake Ontario supports this view. Sensitivity to both PAR and UVR was far greater during spring overturn than in summer (Smith et al. 1998). Further support can be found in the results of the the whole-lake acidification experiment of Lake 302 (Experimental Lakes Area, northwestern Ontario) (Leavitt et al. 1999), where the pH reduction induced strong UVR effects. DOC concentration (Schindler et al. 1996) and quality (Donahue et al. 1998) were reduced when pH fell, resulting in elevated UVR penetration. As a result, sediments of Lake 302 contained significant concentrations of pigments characteristic of UVR-transparent environments (Leavitt et al. 1999). Associated with these changes, there was an increase in the relative importance of dinoflagellates and chlorophytes at the expense of chrysophytes and diatoms (naturally present in the lake before acidification). The abundance of dinoflagellates was more strongly correlated with the depth of UVR penetration ($r^2 = 0.90$) than with pH ($r^2 = 0.68$) (Leavitt et al. 1999).

Thus the natural ozone-thinning cycle that leads from a spring maximum to a fall minimum in the Northern Hemisphere may contribute to taxonomic changes in lakes that have previously been assigned only to nutrient dynamics, temperature, and grazing. Furthermore, it follows that the sporadic occurrence during the growing season of rapid declines and increases in ozone thickness could contribute to the occurrence of sudden algal blooms. This influence would previously have been undetected; this study produced the first data, to our knowledge, that allow the relationship of community dynamics to UVB to be seen in a natural system. The relationship appears strong, and very logical in hindsight. Without direct measurements of UVB radiation, we would not have been aware of the large week-to-week and even day-to-day variations in UVR incident energy that appear to be important to lake communities.

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References

ACGIH-NIOSH. 1972. Criteria for a recommended standard occupational exposure to ultraviolet radiation. Document 73-11009, NIOSH, Centers for Disease Control, Atlanta, Ga. (Website: http://www.cdc.gov/niosh/73-11009.html)

Bothwell, M.L., Sherbot, D.M.J., and Pollock, C.M. 1994. Ecosystem response to solar ultraviolet-B radiation: influence of trophic-level interactions. Science (Washington, D.C.), **265**: 97–100.

Butow, B., Wynne, D., Sukenik, A., Hadas, O., and Tel-Or, E. 1998. The synergistic effect of carbon concentration and high temperature on lipid peroxidation in *Peridinium gatunense*. J. Plankton Res. **20**: 355–369.

Carreto, J.I., Carignan, M.O., Daleo, G., and De Marco, S.G. 1990. Occurrence of mycosporine-like amino acids in the red tide dinoflagellate *Alexandrium excavatum*: UV-photoprotective compounds? J. Plankton Res. **12**: 909–921.

Cooper, W.J., and Zika, R.G. 1983. Photochemical formation of hydrogen peroxide in surface and ground waters exposed to sunlight. Science (Washington, D.C.), **220**: 711–712.

Cullen, J.J., and Lesser, M.P. 1991. Inhibition of photosynthesis by ultraviolet radiation as a function of dose and dosage rate: results for a marine diatom. Mar. Biol. **111**: 183–190.

Cullen, J.J., Neale, P.J., and Lesser, M.P. 1992. Biological weighting function for the inhibition of phytoplankton photosynthesis by ultraviolet radiation. Science (Washington, D.C.), **258**: 646–650.

Davidson, A.T., Marchant, H.J., and De La Mare, W.K. 1996. Natural UVB exposure changes the species composition of Antarctic phytoplankton in mixed culture. Aquat. Microb. Ecol. **10**: 299–305.

Donahue, W.F., Schindler, D.W., Page, S.J., and Stainton, M.P. 1998. Acid-induced changes in DOC quality in an experimental whole-lake manipulation. Environ. Sci. Technol. **32**: 2954–2960.

Edouard, S., Legras, B., Lefèvre, F., and Eymard, R. 1996. The effect of small-scale inhomogeneities on ozone depletion in the Arctic. Nature (Lond.), **384**: 444–447.

Ekelund, N.G.A. 1990. Effects of UV-B radiation on growth and motility of four phytoplankton species. Physiol. Plant. **78**: 590–594.

Falkowski, P.G., and LaRoche, J. 1991. Acclimation toa spectral irradiance in algae. J. Phycol. **27**: 8–14.

Garcia-Pichel, F., and Castenholz, R.W. 1991. Characterization and biological implications of scytonemin, a cyanobacterial sheath pigment. J. Phycol. **27**: 395–409.

Helbling, E.W., Villafane, V., Ferrario, M., and Holm-Hansen, O. 1992. Impact of natural ultraviolet radiation on rates of photosynthesis and on specific marine phytoplankton species. Mar. Ecol. Prog. Ser. **80**: 89–100.

Hill, W.R., Dimick, S.M., McNamara, A.E., and Branson, C.A. 1997. No effects of ambient UV radiation detected in periphyton and grazers. Limnol. Oceanogr. **42**: 769–779.

Imberger, J. 1985. The diurnal mixed layer. Limnol. Oceanogr. **30**: 737–770.

Jeffrey, W.H., Pledger, R.J., Aas, P., Hager, S., Coffin, R.B., Von Haven, R., and Mitchell, D.L. 1996. Diel and depth profiles of DNA photodamage in bacterioplankton exposed to ambient solar ultraviolet radiation. Mar. Ecol. Prog. Ser. **137**: 283–291.

Jones, R.I. 1993. Phytoplankton migrations: patterns, processes and profits. Ergebn. Limnol. **39**: 67–77.

Kana, T.M., Glibert, P.M., Goericke, R., and Welshmey, N.A. 1988. Zeaxanthin and beta-carotene in *Synechococcus* WH7803 respond differently to irradiance. Limnol. Oceanogr. **33**: 1623–1627.

Karentz, D. 1994. Ultraviolet tolerance mechanisms in Antarctic marine organisms. Antarct. Res. Ser. **62**: 93–110.

Karentz, D., Bothwell, M.L., Coffin, R.B., Hanson, A., Herndl, G.J., Kilham, S.S., Lesser, M.P., Lindell, M., Moeller, R.E., Morris, D.P., Neale, P.J., Sanders, R.W., Weiler, C.S., and Wetzel, R.G. 1994. Impact of UV-B radiation on pelagic freshwater ecosystems: report of working group on bacteria and phytoplankton. Arch. Hydrobiol. Beih. Ergebn. Limnol. **43**: 31–69.

Kerr, J.B., and McElroy, C.T. 1993. Evidence for large upward trends of ultraviolet-B radiation linked to ozone depletion. Science (Washington, D.C.), **262**: 1032–1034.

Kirk, J.T.O. 1994. Optics of UV-B radiation in natural waters. Arch. Hydrobiol. Beih. Ergebn. Limnol. **43**: 1–16.

Laurion, I., Lean, D.T.S., and Vincent, W.F. 1998. UVB effects on a plankton community: results from large-scale enclosure assay. Aquat. Microb. Ecol. **16**: 189–198.

Leavitt, P.R., Findlay, D.L., Hall, R.I., and Smol, J.P. 1999. Algal responses to dissolved organic carbon loss and pH decline during whole-lake acidification evidence from paleolimnology. Limnol. Oceanogr. **44**: 757–773

Lindell, M.J., and Rai, H. 1994. Photochemical oxygen consumption in humic waters. Arch. Hydrobiol. Beih. Ergebn. Limnol. **43**: 145–155.

Madronich, S., McKenzie, R.L., Caldwell, M.M., and Björn, L.O. 1995. Changes in ultraviolet radiation reaching the earth's surface. Ambio, **24**: 143–152.

McKinlay, A.F., and Diffey, B.L. 1987. A reference action spectrum for ultra-violet induced erythema in human skin. *In* Human exposure to ultraviolet radiation: risk and regulations. *Edited by* W.F. Passchier and B.F.M. Bosnjakovit. Excerpta Med. Int. Congr. Ser. 744.

McQueen, D.J., and Lean, D.R.S. 1987. Influence of water temperature and nitrogen to phosphorus ratios in the dominance of blue-green algae in Lake St. George, Ontario. Can. J. Fish. Aquat. Sci. **44**: 598–604.

Milot-Roy, V., and Vincent, W.F. 1994. UV radiation effects on photosynthesis: the importance of near-surface thermoclines in a subarctic lake. Ergebn. Limnol. **43**: 171–184.

Neale, P.J., Davies, R.F., and Cullen, J.J. 1998. Interactive effects of ozone depletion and vertical mixing on photosynthesis of Antarctic phytoplankton. Nature (Lond.), **392**: 585–589.

Nielsen, T., and Ekelund, N.G.A. 1993. Effect of UV-B radiation and humic substances on growth and motility of *Gyrodinium aureolum*. Limnol. Oceanogr. **38**: 1570–1575.

Paerl, H.W., Tucker, J., and Bland, P.T. 1983. Carotenoid enhancement and its role in maintaining blue-green algal (*Microcystis aeruginosa*) surface blooms. Limnol. Oceanogr. **28**: 847–857.

Sartory, D.P., and Grobbelaar, J.U. 1984. Extraction of chlorophyll *a* from freshwater phytoplankton for spectrophotometric analysis. Hydrobiologia, **114**: 177–187.

Schindler, D.W., Bayley, S.E., Parker, B.R., Beaty, K.B., Cruikshank, D.R., Fee, E.J., Schindler, E.U., and Stainton, M.P. 1996. The effects of climatic warming on the properties of boreal lakes and streams at the Experimental Lakes Area, northwestern Ontario. Limnol. Oceanogr. **41**: 1004–1017.

Scully, N.M., and Lean, D.R.S. 1994. The attenuation of ultraviolet radiation in temperate lakes. Ergebn. Limnol. **43**: 135–144.

Scully, N.M., Vincent, W.F., Lean, D.R.S., and MacIntyre, S. 1998. Hydrogen peroxide as a natural tracer of mixing in surface layers. Aquat. Sci. **60**: 169–186.

Setlow, R.B. 1974. The wavelengths in sunlight effective in producing skin cancer: a theoretical analysis. Proc. Natl. Acad. Sci. U.S.A. **71**: 3363–3366.

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Smith, R.E.H., Furgal, J.A., and Lean, D.R.S. 1998. The short-term effects of solar ultraviolet radiation on phytoplankton photosynthesis and photosynthetate allocation under contrasting mixing regimes in Lake Ontario. J. Gt. Lakes Res. **24**: 427–441.

Xenopoulos, M.A., and Bird, D.F. 1997. Effect of acute exposure to hydrogen peroxide on the production of phytoplankton and bacterioplankton in a mesohumic lake. Photochem. Photobiol. **65**: 471–478.

Zhang, Y., and Prepas, E.E. 1996. Regulation of the dominance of planktonic diatoms and cyanobacteria in four eutrophic hard-water lakes by nutrients, water column stability, and temperature. Can. J. Fish. Aquat. Sci. **53**: 621–633.

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Thalassiosira baltica (Grunow) Ostenfeld (Bacillariophyta), a new exotic species in the Great Lakes

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Abstract: Microfossil analysis of sediment cores collected in 1994 and phytoplankton collected in 1998 from Lake Ontario indicated that the diatom *Thalassiosira baltica* (Grunow) Ostenfeld was introduced and became established ca. 1988 according to ^{210}Pb date estimates and has remained a predominant component of the phytoplankton since its establishment. *Thalassiosira baltica* is an exotic species in the Laurentian Great Lakes and is more commonly found in coastal brackish waters in western and northern Europe and only rarely reported from North American coastal waters. The probable vector for introduction of *T. baltica* was ballast water exchange and the successful establishment attributed to this taxon's euryhalinity tolerance.

Résumé : D'après l'analyse des microfossiles contenus dans des carottes de sédiments prélevées en 1994 et du phytoplancton recueilli en 1998 dans le lac Ontario, la diatomée *Thalassiosira baltica* est une espèce introduite; son établissement remonterait à 1988 environ selon les estimations au ^{210}Pb, et elle est depuis un élément prédominant du phytoplancton. Il s'agit d'une espèce exotique dans les Grands Lacs laurentiens, et sa présence est plus fréquente dans les eaux saumâtres côtières de l'ouest et du nord de l'Europe; elle n'a été signalée qu'à de rares occasions dans les eaux côtières d'Amérique du Nord. La vidange des eaux de lest est probablement le vecteur d'introduction de *Thalassiosira baltica*, et l'euryhalinité de ce taxon explique son établissement.

[Traduit par la Rédaction]

Introduction

The introduction of exotic species by intentional or unintentional means is a current and recurrent concern throughout the world (Cohen and Carlton 1998) and especially in the Great Lakes region. Mills et al. (1993) reviewed the history of exotic species introductions in the Great Lakes and reported on the establishment of 139 species since the 1800s. Twenty-four of these introductions were algal species, which included 16 diatom taxa. Most of the diatom taxa were probably of European freshwater origin; however, a portion of the introductions included taxa that were tolerant of salinity, were limited in their introduced distribution to nearshore and polluted regions, and were probably of coastal or estuarine origin (Mills et al. 1993). Whereas many of the introduced primary producers have had little or no measurable impact on the Great Lakes ecosystem, the list of introduced algae does include biofouling (e.g., *Bangia fuscopurpurea* (Dillwyn) Lyngb. (as *Bangia atropurpurea* (Roth) C.Agardh); Lin and Blum 1977) and taste- and odor-causing forms (e.g., *Stephanodiscus binderanus* (Kütz.) Willi Krieg.; Brunel 1956; Vaughn 1961). Additionally, the temporal relationships between introductions of exotic species and extirpations of Great Lakes native diatoms suggest that some impacts may not have been fully accounted (Stoermer et al. 1985; Julius et al. 1998).

Only minimal efforts are currently being made to monitor seasonal and interannual changes in offshore Great Lakes primary producer communities. Recent monitoring efforts have been unable to resolve significant interannual trends in offshore phytoplankton, even though important changes such as reductions in phosphorus loadings have been documented (Nicholls and Hopkins 1988); in contrast, monitoring of nearshore chemical and biotic conditions has indicated some primary producer response to decreased phosphorus loadings and exotic introductions (Nicholls and Hopkins 1993). Shortcomings in our ability to monitor and resolve changes suggest that a paleolimnological analysis is a most feasible approach to analyzing recent responses by the Great Lakes primary producer community to both remediation efforts and continued ecological perturbation. Variability in response to these influences is often difficult to interpret at small temporal and large areal scales, but by studying sedimentary remains, spatial and seasonal variations are integrated, thus revealing both short- (annual) and long-term (decades to centuries) system responses (Wolin et al. 1991; Stoermer et al. 1996; Hairston et al. 1999). This approach has been used successfully in all of the Great Lakes (Stoermer et al. 1993) to identify historical changes in plankton communities and has shown that introduced primary producers were often present in the system well before they were detected by

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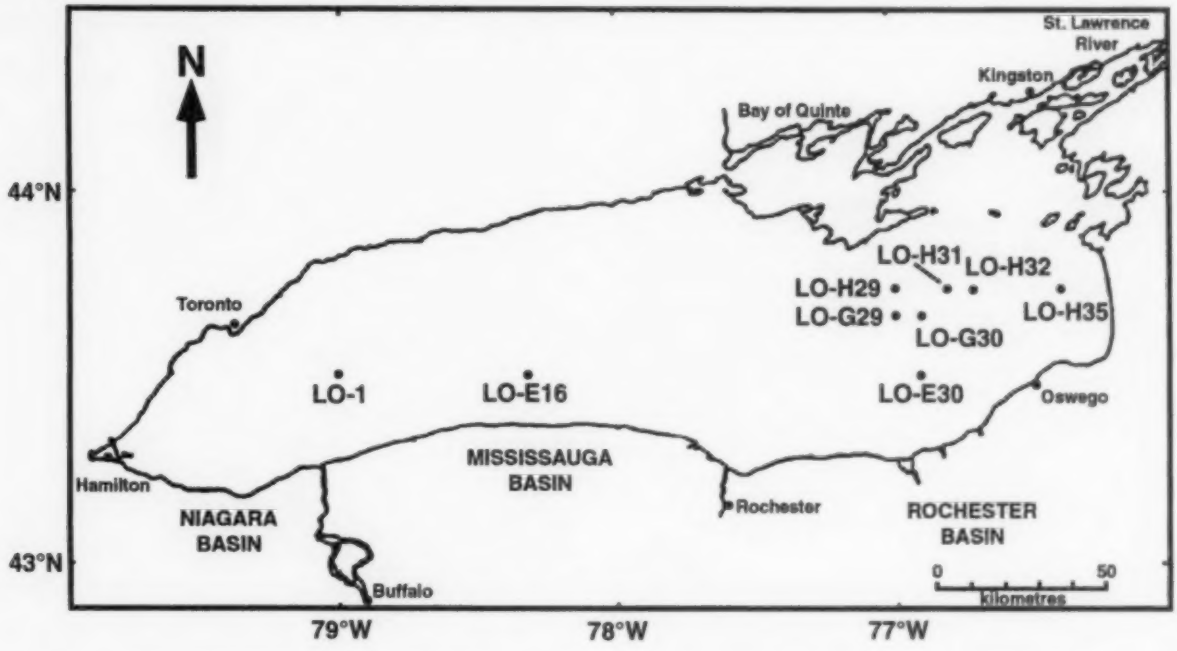
Fig. 1. Map of Lake Ontario showing the locations where core LO-H32-94 was recovered and where surficial sediments were collected in 1994. LO-1 (43°30.0′N, 79°00.0′W), LO-E16 (43°30.0′N, 78°18.0′W), LO-E30 (43°30.0′N, 76°54.0′W), LO-G29 (43°38.6′N, 77°00.0′W), LO-G30 (43°38.6′N, 76°54.0′W), LO-H29 (43°42.9′N, 77°00.0′W), LO-H31 (43°42.9′N, 76°48.0′W), LO-H32 (43°42.9′N, 77°42.0′W), and LO-H35 (43°42.9′N, 76°24.0′W).



monitoring efforts (Hohn 1951; Brunel 1956; Stoermer et al. 1985). Similarly, this analysis of recent cores, surficial sediment, and plankton collections from Lake Ontario has documented the introduction (ca. 1988) and establishment of a new exotic diatom species, *Thalassiosira baltica* (Grunow) Ostenfeld.

Materials and methods

Sediment core LO-H32-94 was retrieved from Rochester Basin in eastern Lake Ontario (43°42.9′N, 76°42.0′W) (Fig. 1) on 25 May 1994 using a Soutar box corer (0.5 × 0.5 × 1.0 m) and sampled according to the methods described in Schelske and Hodell (1991) at 0.5-cm increments from the sediment–water interface to 20 cm depth and at 1.0-cm increments from 20 cm to the base of the section (80 cm). Samples were frozen in the field, freeze-dried in the laboratory, and stored (Department of Fisheries and Aquatic Sciences, University of Florida, Gainesville, Fla.) for further analyses.

For quantitative analysis, approximately 25 mg of sediment was processed using the cleaning methods of Stoermer et al. (1995). Cleaned material was placed in Battarbee chambers (Battarbee 1973), allowed to dry overnight at 50°C in a Fisher Isotemp oven, and the four resultant coverslips with randomly distributed microfossils mounted with Naphrax (Northern Biological Supplies) on microscope slides for light microscopy. Siliceous microfossils were enumerated using oil immersion on a Leitz Ortholux light microscope at 950× magnification. A minimum of 500 diatom valves was counted from one slide per sampling interval unless the abundance was too low (< 500 valves per coverslip), in which case the entire coverslip was counted. All siliceous microfossils (sponge spicules, chrysophyte cysts, and diatom valves, including fragments) encountered were enumerated and raw counts converted to diatom abundance as valves per gram dry weight of sediment and relative abundance as valves relative to total siliceous microfossils. Duplicate slides of material from core LO-H32-94 have been deposited in the Diatom Collection at the California Academy of Sciences (San Francisco, Calif.).

Morphometric parameters of *T. baltica* valves including valve diameter, areolae density in 10 μm at midradius, and number of rimoportulae were measured using a Leitz DMRX light microscope and the digitizing and computer setup described by Stoermer (1996). Additional coverslips with material were attached to aluminum stubs, coated with 25 nm of Au–Pd, and viewed at an accelerating voltage of 25 kV in a JEOL JSM-T100 scanning electron microscope. Identification of *T. baltica* followed Hasle (1978) and Krammer and Lange-Bertalot (1991).

The core was dated using radiometric data obtained by low-background gamma spectrometry (Schelske et al. 1994). Sediment ages were calculated on samples with excess ^{210}Pb activity using the constant rate of supply model (Appleby and Oldfield 1978). Supported ^{210}Pb was estimated from ^{226}Ra activity. Very precise dates are generated by the age model, and, realistically, the dating error, based on the mean life of ^{210}Pb, is no greater than 2 or 3 years. Measurements of ^{137}Cs activity provided an independent age marker of the 1962–1963 peak in fallout radioactivity. The ^{137}Cs peak was at 17.75 cm (17.5 and 18.0 cm values were essentially equal). The marker date for this peak agrees with the ^{210}Pb date for these samples.

Results

Paleolimnological analysis of a ^{210}Pb-dated core from Lake Ontario showed that *T. baltica* was first evident in quantifiable amounts in Lake Ontario from sediments depos-

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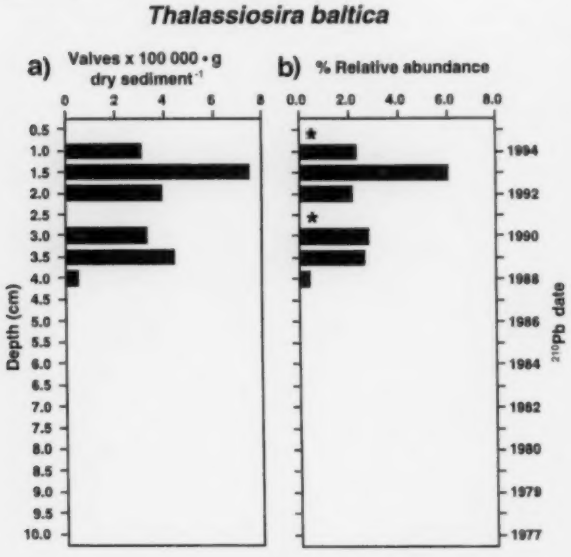
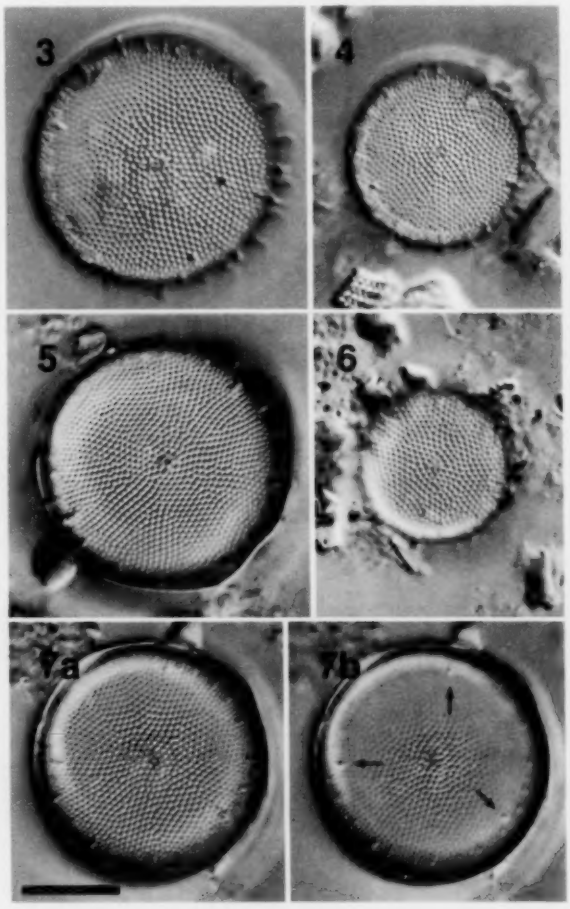
Fig. 2. Depth profile of (*a*) absolute abundance and (*b*) relative abundance of *T. baltica* valves in core LO-H32-94. The left abscissa shows absolute depth in the core; the right abscissa shows ^{210}Pb dates of selected levels rounded to the nearest year. Asterisks at two depths indicate the presence of *T. baltica* in sediments but at relative abundance below 0.2%.



ited ca. 1988 (Fig. 2). In sediments deposited ca. 1992, *T. baltica* had increased to nearly 6% relative abundance within the preserved assemblage (Fig. 2). Whereas the surficial sediments in core LO-H32-94 did not contain quantifiable remains of *T. baltica* (Fig. 2), extended counts of microscope slides (>500 valves) and analysis of collections of surficial sediments taken in 1994 from Lake Ontario did reveal its continued presence throughout the lake (Fig. 1). Variability in sedimentary abundances may be reflecting seasonal or annual productivity differences. In addition, live cells of *T. baltica* were encountered in Lake Ontario phytoplankton net collections made in 1998 (unpublished observations).

Cells of *T. baltica* from Lake Ontario are discoid cylinders with flat or slightly concave valve faces that meet shallow mantles at an obtuse angle (Fig. 8). Valves are 15.8–27.0 μm in diameter (mean 21.8 μm, n = 50). Valve faces and mantle are covered with loculate areolae (Figs. 8–11) arranged on the face in sectored radial rows running parallel to a radius (Figs. 3–7), with midradius densities of 12.7–17.8 areolae in 10 μm (mean 15.6 areolae, n = 50). Areolae are more densely packed on the margin and mantle of the valve (Fig. 8). External openings of areolae are simple pores with or without volate occlusions; internal areolar openings are occluded by flat to internally domed cribra. Each valve has two or three rimoportulae (labiate processes) located at junction of the valve face and mantle. External expression of each rimoportula is a conical tube with or without upturned end (Figs. 8 and 10) and internal expression is sessile or with a short stalk (Figs. 9 and 11). Four to six central fultoportulae are located in the middle of the valve face. External expression of each central fultoportula is a simple

Figs. 3–7. Differential interference light micrographs showing size diminution series of *T. baltica*. Fig. 7. (*a*) High focus on valve face; (*b*) midfocus showing three rimoportulae (arrows). Scale bar for all figures (shown in Fig. 7*a*) = 10 μm.



pore and the internal tube is surrounded by four cowlings (Figs. 8 and 11). Two rings of marginal fultoportulae (strutted processes) are located on the valve mantle (Figs. 8–11). Internal expressions of both rings of marginal fultoportulae are similar with four cowlings surrounding each internal tube (Figs. 9 and 11). The inner ring of marginal fultoportulae has larger external expressions than the outer ring (Figs. 8 and 10). External expressions of the inner ring of marginal fultoportulae are funnelform to doliform with oblique openings and a fluted basal region; external expressions of the outer marginal fultoportula ring are fluted tubes to funnelform projections (Figs. 8 and 10).

Discussion

Specimens of *T. baltica* from Lake Ontario generally correspond to published descriptions (Hasle 1978; Krammer and Lange-Bertalot 1991; Semina and Fryxell 1994). Lake Ontario specimens, however, overlapped the low end of the published size and rimoportulae range noted by other au-

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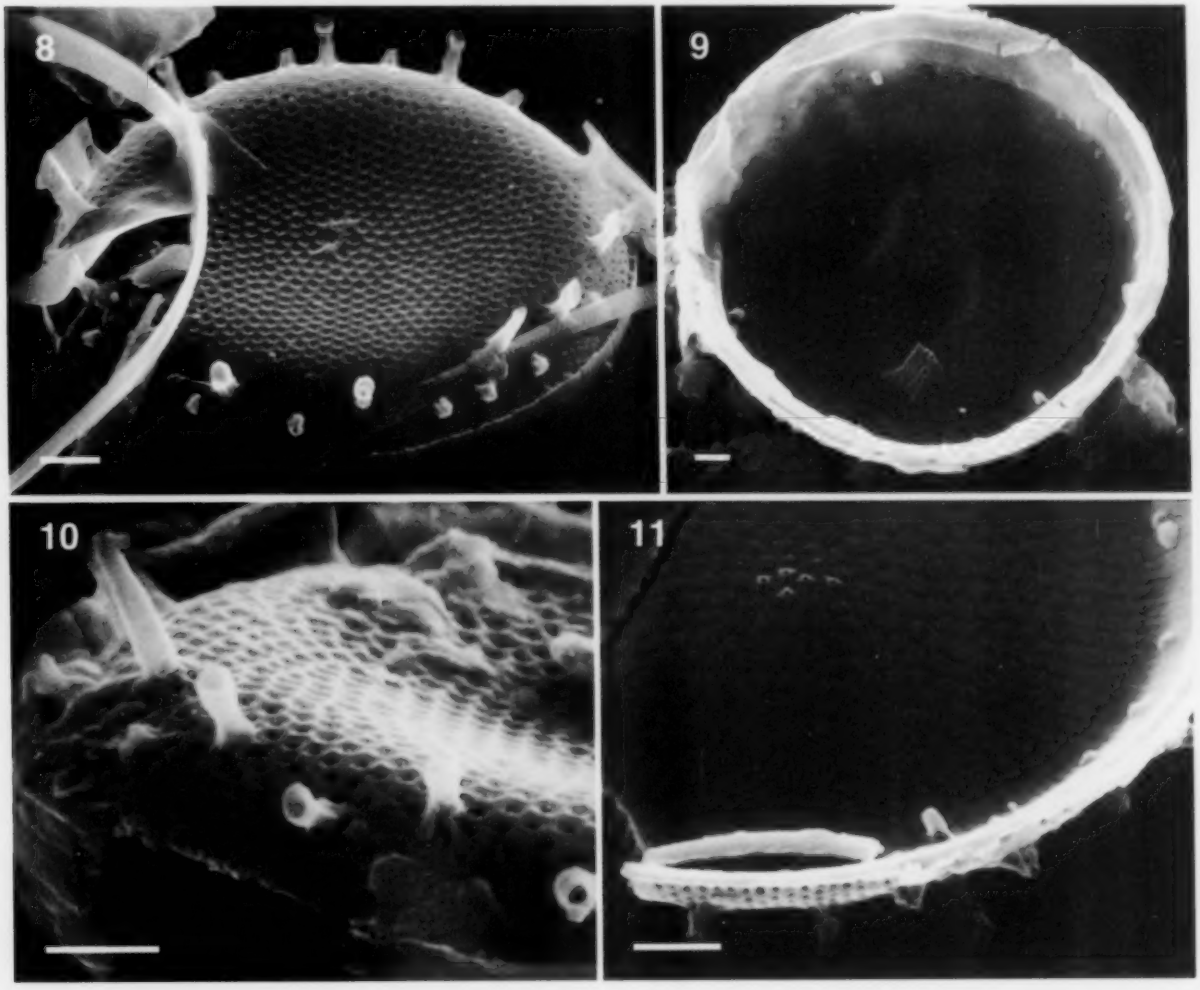
Figs. 8–11. Scanning electron micrographs of *T. baltica*. Fig. 8. External view of valve. Fig. 9. Internal view of valve showing sectored fasciculate arrays of areolae, double ring of marginal fultoportulae, and three rimoportulae. Fig. 10. External mantle area. Note the tapered tubular external extension of rimoportula and funnelform external expressions atop fluted bases on the two rings of marginal fultoportulae. Fig. 11. Interior of valve showing flat internal cribra, cluster of five central fultoportulae, each with four satellite pores, internal and external expression of rimoportula, and internal and external expression of two rings of marginal fultoportulae. Scale bars in all figures = 2 μm.



thors. Hasle (1978) reported *T. baltica* to range from 20 to 120 μm in diameter and to have three to five rimoportulae, whereas Lake Ontario specimens were as small as 15.8 μm in diameter and had two or three rimoportulae. Small-sized specimens may account for the discrepancy in rimoportulae count; other taxa are known to have similar morphometric relationships (e.g., *Stephanodiscus niagarae* Ehrenb.; Theriot and Stoermer 1984). Other morphological parameters noted in Lake Ontario populations of *T. baltica* were in agreement with published accounts.

Eight other *Thalassiosira* taxa have been reported from the Great Lakes (Stoermer et al. 1999) including *Thalassiosira bramaputrae* (Ehrenb.) Håk. & Locker (synonym: *Thalassiosira lacustris*), *Thalassiosira weissflogii* (Grunow) G.A. Fryxell & Hasle (synonym: *Thalassiosira fluviatilis*), *Thalassiosira incerta* I.V.Makarova, *Thalassiosira guillardii* Hasle, *Thalassiosira levanderi* Goor, *Thalassiosira simplex* Hust., *Thalassiosira pseudonana* Hasle & Heimdal, and *Thalassiosira visurgis* Hust. *Thalassiosira baltica* differs from *T. bramaputrae* in that it has a flat valve face compared with a tangentially undulate face on *T. bramaputrae*. *Thalassiosira weissflogii*, *T. incerta*, *T. guillardii*, *T. pseudonana*, and *T. simplex* have only a single rimoportula compared to two or three rimoportulae in Lake Ontario populations of *T. baltica*. *Thalassiosira baltica* also differs in that *T. levanderi* is small (13–20 μm in diameter) and has areolae arranged in eccentric curved rows, and *T. visurgis* has a coarsely punctate valve face with areolae not arranged in radial sectors. The only other common Great Lakes diatom with which *T. baltica* might be confused is *Actinocyclus*

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normanii fo. *subsalsa* (Juhl.-Dannf.) Hust.; however, the former has fewer and smaller rimoportulae and possesses marginal fultoportulae that appear spinelike, which are absent in the latter.

Thalassiosira baltica has many autecological characteristics that may have contributed to its success as an invader in the Great Lakes ecosystem. Ricciardi and Rasmussen (1998) outlined 11 attributes of successful invasive aquatic species, and among these, *T. baltica* is widely distributed and has wide environmental tolerances, short generation times, rapid growth, the capability of rapid dispersal and is commensal with human activity (Hasle 1978; Krammer and Lange-Bertalot 1991). Notable among these attributes for invasion are the known distribution of *T. baltica* and its euryhalinity tolerance. *Thalassiosira baltica* is found in spring collections from coastal European waters, polar seas, and in the large inland seas of Eurasia (Hasle 1978), making its potential for transoceanic ballast water transport very high. Whereas we have not tested molecular markers of origin, Eurasian waters are the probable site of origin for *T. baltica*, as it has only been rarely reported in Western Hemisphere waters (Hasle 1978; Cooper 1995). *Thalassiosira baltica* has also been reported from waters with salinities of 4.8–44‰ (Hasle 1978), increasing its probability of surviving ballast water exchange programs. Lake Ontario represents the freshest water where *T. baltica* has been reported (conductivity approximately 300 $\mu mho \cdot cm^{-1}$; Johannsson et al. 1998), indicating that *T. baltica* is even more tolerant of, or has developed tolerance to, lower salinities than previously thought. Interestingly, other marine taxa that have become established in freshwater systems have also been reported to have smaller cell sizes than their marine populations (Lowe and Busch 1975), perhaps illustrating a physiological adaptation to freshwater environments. While *T. baltica* is not known to undergo resting spore formation, it may be able to form resting cells capable of long-term survival in dark, anoxic environments, similar to other members of the family Thalassiosiraceae (Doucette and Fryxell 1983; Sicko-Goad et al. 1989).

Whereas invasive higher organisms usually receive greater public attention as a benefit of popular press, most of the lower organismal groups also contain successful invaders (Mills et al. 1993). On a worldwide basis, reports of exotic diatoms include both freshwater (Harper 1994) and marine introductions (Anderson and Garrison 1997) with consequences as dramatic as human and shellfish poisonings by domoic acid producers. In the Laurentian Great Lakes, 16 diatom taxa are considered past invaders (Mills et al. 1993). Of these, all were probable ballast water introductions and the list contains both freshwater and brackish/marine representatives. Several of the brackish/marine introductions include taxa that have had limited temporal establishment in the Great Lakes and (or) have been restricted to areas with elevated total dissolved solids (e.g., *T. visurgis*, *T. weissflogii*, *Pleurosira laevis* (Ehrenb.) Compère, *Terpsinoe musica* Ehrenb.; Stoermer and Yang 1969; Wujek and Welling 1981). Other introductions include cosmopolitan freshwater species that were absent from the Great Lakes before European colonization and may have also responded favorably to post-European changes in nutrient dynamics of the system (e.g., *Cyclotella pseudostelligera* Hust., *Stephanodiscus subtilis* (Goor) A.Cleve, *S. binderanus*; Stoermer and Yang 1969; Mills et al. 1993).

The future impact of *T. baltica* on the Great Lakes ecosystem is difficult to predict. Impacts from earlier exotic diatom introductions have similarly not been well documented. The most noticeable impact followed the establishment of *S. binderanus* in the Great Lakes; it was subsequently shown to be a filter-clogging organism and the cause of taste and odor problems (Brunel 1956; Vaughn 1961). The only other probable impact of exotic diatoms is from *A. normanii* fo. *subsalsa*. This taxon has a very heavily silicified wall and has its major growth period in summer. *Actinocyclus normanii* fo. *subsalsa* may have been important in depleting dissolved silica in shallow bays and nearshore areas and thus promoted the growth of blue-green algae (Theriot and Stoermer 1985). The impact of diatom introductions may often be more subtle than these noticeable conditions. One potential outcome of introductions is the extirpation of native taxa via competition. Some evidence to suggest that this has occurred, or at least that introductions have been concomitant with extirpations, has been presented. Stoermer et al. (1985) analyzed a sediment core from Lake Ontario and showed that the introductions of *S. binderanus* and *A. normanii* fo. *subsalsa* in Lake Ontario were concomitant with local extinctions of *Stephanodiscus transilvanicus* Pant., *Cyclotella comta* (Ehrenb.) Kütz., *Cyclotella michiganiana* Skvortsov, *Cyclotella ocellata* Pant., and *Cyclotella stelligera* (Cleve & Grunow) Van Heurck from that system. More recently, Julius et al. (1998) showed that *S. niagarae* has been extirpated from its type locality, Lake Ontario, almost concomitant with the introduction and establishment of *T. baltica*. Whether *T. baltica* caused the extirpation of *S. niagarae* or only if modern conditions in Lake Ontario were able to allow the colonization of *T. baltica* remains to be resolved.

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References

Anderson, D.M., and Garrison, D.J. (*Editors*). 1997. The ecology and oceanography of harmful algal blooms. Limnol. Oceanogr. **42**: 1009–1305.

Appleby, P.G., and Oldfield, F. 1978. The calculation of ^{210}Pb dates assuming a constant rate of supply of unsupported ^{210}Pb to the sediment. Catena, **5**: 1–8.

Battarbee, R.W. 1973. A new method for estimating absolute microfossil numbers with special reference to diatoms. Limnol. Oceanogr. **18**: 647–653.

Brunel, J. 1956. Addition du *Stephanodiscus binderanus* à la flore diatomique de l'Amérique du Nord. Nat. can. (Qué.), **83**: 91–95.

Cohen, A.N., and Carlton, J.T. 1998. Accelerating invasion rate in a hightly invaded estuary. Science (Washington, D.C.), **279**: 555–557.

Cooper, S.R. 1995. Diatoms in sediment cores from the mesohaline Chesapeake Bay, U.S.A. Diatom Res. **10**: 39–89.

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Doucette, G.J., and Fryxell, G.A. 1983. *Thalassiosira antarctica*: vegetative and resting stage chemical composition of an ice-related marine diatom. Mar. Biol. (Berl.), **78**: 1–6.

Hairston, N.G., Jr., Perry, L.J., Bohonak, A.J., Fellows, M.Q., Kearns, C.M., and Engstrom, D.R. 1999. Population biology of a failed invasion: paleolimnology of *Daphnia exilis* in upstate New York. Limnol. Oceanogr. **44**: 477–486.

Harper, M.A. 1994. Did Europeans introduce *Asterionella formosa* Hassall to New Zealand? *In* Proceedings of the Eleventh International Diatom Symposium. *Edited by* J.P. Kociolek. Mem. Calif. Acad. Sci. No. 17. California Academy of Sciences, San Francisco, Calif. pp. 479–484.

Hasle, G.R. 1978. Some freshwater and brackish water species of the diatom genus *Thalassiosira* Cleve. Phycologia, **17**: 263–292.

Hohn, M.H. 1951. A study of the distribution of diatoms (Bacillarieae) in western New York State. Cornell Univ. Agric. Exp. Stn. Mem. 308.

Johannsson, O.E., Millard, E.S., Ralph, K.M., Myles, D.D., Graham, D.M., Taylor, W.D., Giles, B.G., and Allen, R.E. 1998. The changing pelagia of Lake Ontario (1981 to 1995): a report of the DFO Long-Term Biomonitoring (Bioindex) Program. Can. Tech. Rep. Fish. Aquat. Sci. No. 2243.

Julius, M.L., Stoermer, E.F., Taylor, C.M., and Schelske, C.L. 1998. Local extirpation of *Stephanodiscus niagarae* Ehrenb. (Bacillariophyta) in the recent limnological record of Lake Ontario. J. Phycol. **34**: 766–771.

Krammer, K., and Lange-Bertalot, H. 1991. Bacillariophyceae. 3. Teil: Centrales, Fragilariaceae, Eunotiaceae. *In* Süsswasserflora von Mitteleuropa, Band 2/3. *Edited by* H. Ettl, J. Gerloff, H. Heynig, and D. Mollenhauer. Gustav Fischer Verlag, Stuttgart, Jena.

Lin, C.K., and Blum, J.L. 1977. Recent invasion of a red alga (*Bangia atropurpurea*) in Lake Michigan. J. Fish. Res. Board Can. **34**: 2413–2416.

Lowe, R.L., and Busch, D.E. 1975. Morphological observations on two species of the diatom genus *Thalassiosira* from fresh-water habitats in Ohio. Trans. Am. Microsc. Soc. **94**: 118–123.

Mills, E.L., Leach, J.H., Carlton, J.T., and Secor, C.L. 1993. Exotic species in the Great Lakes and anthropogenic introductions. J. Gt. Lakes Res. **19**: 1–54.

Nicholls, K., and Hopkins, G. 1988. Lake Ontario north shore phytoplankton and nutrient trends (municipal water intake monitoring). *In* Rehabilitation of Lake Ontario: the role of nutrient reduction and food web dynamics. Report to the Great Lakes Science Advisory Board, International Joint Commission, Great Lakes Regional Office, Windsor, Ont.

Nicholls, K.H., and Hopkins, G.J. 1993. Recent changes in Lake Erie (north shore) phytoplankton: cumulative impacts of phosphorus loading reductions and the zebra mussel introduction. J. Gt. Lakes Res. **19**: 637–647.

Ricciardi, A., and Rasmussen, J.B. 1998. Predicting the identity and impact of future biological invaders: a priority for aquatic resource management. Can. J. Fish. Aquat. Sci. **55**: 1759–1765.

Schelske, C.L., and Hodell, D.A. 1991. Recent changes in productivity and climate of Lake Ontario detected by isotopic analysis of sediments. Limnol. Oceanogr. **36**: 961–975.

Schelske, C.L., Peplow, A., Brenner, M., and Spencer, C.N. 1994. Low-background gamma counting: applications for ^{210}Pb dating of sediments. J. Paleolimnol. **10**: 115–128.

Semina, H.J., and Fryxell, G.A. 1994. *Thalassiosira echinata* Semina, a new diatom species from the equatorial Pacific. Diatom Res. **9**: 377–385.

Sicko-Goad, L., Stoermer, E.F., and Kociolek, J.P. 1989. Diatom resting cell rejuvenation and formation: time course, species records and distribution. J. Plankton Res. **11**: 375–389.

Stoermer, E.F. 1996. A simple, but useful, application of image analysis. J. Paleolimnol. **15**: 111–113.

Stoermer, E.F., and Yang, J.J. 1969. Plankton diatom assemblages in Lake Michigan. Gt. Lakes Res. Div. Spec. Rep. No. 47. University of Michigan, Ann Arbor, Mich.

Stoermer, E.F., Wolin, J.A., Schelske, C.L., and Conley, D.J. 1985. An assessment of ecological changes during the recent history of Lake Ontario based on siliceous algal microfossils preserved in sediments. J. Phycol. **21**: 257–276.

Stoermer, E.F., Wolin, J.A., and Schelske, C.L. 1993. Paleolimnological comparison of the Laurentian Great Lakes based on diatoms. Limnol. Oceanogr. **38**: 1311–1316.

Stoermer, E.F., Edlund, M.B., Pilskaln, C.H., and Schelske, C.L. 1995. Siliceous microfossil distribution in the surficial sediments of Lake Baikal. J. Paleolimnol. **14**: 69–82.

Stoermer, E.F., Emmert, G., Julius, M.L., and Schelske, C.L. 1996. Paleolimnologic evidence of rapid recent change in Lake Erie's trophic status. Can. J. Fish. Aquat. Sci. **53**: 1451–1458.

Stoermer, E.F., Kreis, R.G., Jr., and Andresen, N.A. 1999. Checklist of diatoms from the Laurentian Great Lakes. II. J. Gt. Lakes Res. **25**: 515–566.

Theriot, E.C., and Stoermer, E.F. 1984. Principal components analysis of character variation in *Stephanodiscus niagarae* Ehrenb.: morphological variation related to lake trophic status. *In* Proceedings of the Seventh International Diatom Symposium. *Edited by* D.G. Mann. Koeltz, Koenigstein. pp. 97–111.

Theriot, E.C., and Stoermer, E.F. 1985. Phytoplankton distribution in Saginaw Bay. J. Gt. Lakes Res. **11**: 132–142.

Vaughn, J.C. 1961. Coagulation difficulties of the South District Filtration Plant. Pure Water, **13**: 45–49.

Wolin, J.A., Stoermer, E.F., and Schelske, C.L. 1991. Recent changes in Lake Ontario 1981–1987: microfossil evidence of phosphorus reduction. J. Gt. Lakes Res. **17**: 229–240.

Wujek, D.E., and Welling, M.L. 1981. The occurrence of two centric diatoms new to the Great Lakes. J. Gt. Lakes Res. **7**: 55–56.

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Assessing covariability among populations in the presence of intraseries correlation: Columbia River spring–summer chinook salmon (*Oncorhynchus tshawytscha*) stocks

Louis W. Botsford and Charles M. Paulsen

Abstract: We assessed covariability among a number of spawning populations of spring–summer run chinook salmon (*Oncorhynchus tshawytscha*) in the Columbia River basin by computing correlations among several different types of spawner and recruit data. We accounted for intraseries correlation explicitly in judging the significance of correlations. To reduce the errors involved in computing effective degrees of freedom, we computed a generic effective degrees of freedom for each data type. In spite of the fact that several of these stocks have declined, covariability among locations using several different combinations of spawner and recruitment data indicated no basinwide covariability. There was, however, significant covariability among index populations within the three main subbasins: the Snake River, the mid-Columbia River, and the John Day River. This covariability was much stronger and more consistent in data types reflecting survival (e.g., the natural logarithm of recruits per spawner) than in data reflecting abundance (e.g., spawning escapement). We also tested a measure of survival that did not require knowing the age structure of spawners, the ratio of spawners in one year to spawners 4 years earlier. It displayed a similar spatial pattern.

Résumé : Nous avons évalué la covariabilité d'un certain nombre de populations reproductrices de quinnat de la remonte de printemps et d'été dans le bassin du Columbia en traitant les corrélations entre plusieurs types de données sur les géniteurs et les recrues. Nous avons explicitement pris en compte la corrélation à l'intérieur des séries pour estimer le caractère significatif des corrélations. Afin de réduire les erreurs dans le calcul des degrés de liberté efficaces, nous avons calculé un degré de liberté efficace générique pour chaque type de données. Plusieurs de ces stocks ont diminué, mais la comparaison entre les lieux établie à l'aide de diverses combinaisons de données sur les géniteurs et le recrutement n'a montré aucune covariabilité à l'échelle du bassin. La covariabilité était cependant importante entre les populations indicatrices des trois principaux sous-bassins suivants : rivière Snake, cours moyen du Columbia et rivière John Day. Cette covariabilité était beaucoup plus forte et plus uniforme dans les types de données touchant la survie (p. ex. logarithme naturel des recrues par géniteur) que dans les données touchant l'abondance (p. ex. échappée de géniteurs). Nous avons également testé une mesure de la survie pour laquelle il n'était pas nécessaire de connaître la structure par âge des géniteurs, le rapport entre le nombre de géniteurs d'une année par rapport à celui des géniteurs quatre ans plus tôt. Cette mesure présentait un profil spatial similaire.

[Traduit par la Rédaction]

Introduction

Covariability between abundance at different locations is a valuable source of information regarding the dynamic structure and physical forcing of spatially distributed populations (e.g., Campbell and Mohn 1983; Koslow et al. 1987; Cohen et al. 1991; Elner and Campbell 1991). Statistical description of the observed covariability over space provides the basis for formulating hypotheses that can then be tested through further analyses and specific measurements or experiments. Such analyses have employed a variety of methods and types of data. Here, we describe covariability among stocks distributed over space by computing correlations between abundance time series. We take special care to account for the effects of covariability within series on the significance of correlations between series. The specific application is to spring–summer runs of chinook salmon (*Oncorhynchus tshawytscha*) in the Columbia River basin (Fig. 1).

A number of stocks of spring–summer chinook salmon on the Columbia River have declined in the past 20 years, and several of them are listed as threatened under the U.S. Endangered Species Act. Management to reverse these declines requires quantitative description of the cause(s) of the decline. Description of causes and evaluation of the effectiveness of various management approaches are the primary goals of the Program for the Analysis and Testing of Hypotheses (PATH). PATH has taken a multipronged approach to these questions using a variety of methods and data

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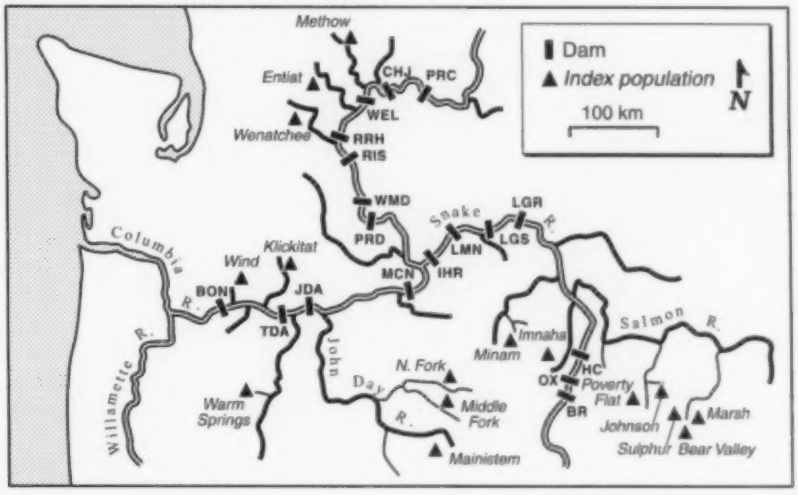
Fig. 1. Map of index streams for stocks of spring–summer chinook salmon analyzed herein. The indicated dams are Bonneville (BON), Dalles (TDA), John Day (JDA), McNary (MCN), Ice Harbor (IHR), Lower Monumental (LMN), Little Goose (LGS), Lower Granite (LGR), Hells Canyon (HC), Oxbow (OX), Brownlee (BR), Priest Rapids (PRD), Wanupum (WMD), Rock Island (RIS), Rocky Reach (RRH), Wells (WEL), Chief Joseph (CHJ), and Grand Coulee (GRC).



sources (e.g., see Shaller et al. (1999) for a parallel PATH analysis). The analysis described here is an initial exploratory part of that process. Here, we test the hypothesis that there has been a similar trend in the state indicators for spring–summer run chinook salmon populations throughout the Columbia River basin. The management implications of the hypothesis are as follows. If all stocks (listed and otherwise) show similar patterns in escapement, recruitment, or other state variables, then some common forcing function (e.g., ocean conditions) would be the most likely candidate for the (joint) decline. On the other hand, if there are systematic differences among races, deems, subbasins or stocks, then causal mechanisms on smaller spatial scales are more likely to be associated with the observed historical patterns.

Methods

Detecting covariability

Covariability between time series can be detected by a variety of statistical methods (e.g., regression, various forms of nonparametric correlation). The various methods of estimating covariability are subject to a number of common problems: intraseries correlation, multiplicity of tests, use of proxy variables, introduction of covariability in preprocessing, and others (Kope and Botsford 1990; Botsford and Brittnacher 1992). Here, we use one of the most common descriptors of covariability, the Pearson product-moment correlation coefficient. Using this statistic to detect covariability between time series involves computing the correlation between the series and then testing to determine whether that value is statistically significant, i.e., whether it is large enough that there is a low probability that it arose from chance alone. We account for problems that arise from intraseries correlation to determine the relative level of confidence that we can have in each specific result, but we do not deal with the other problems that are more or less common to all of the computed correlations. We view the results as identification of pattern rather than a proof of causality. We use a probability level of 0.05 and assume that the correlation coefficient has a Gaussian distribution when it is near zero.

A number of different expressions have been used to estimate the variance of the estimated correlation coefficient in the presence of intraseries correlation (Bayley and Hammersley 1946; Chelton 1983, 1984; Drinkwater and Myers 1987; Kope and Botsford 1990; Botsford and Brittnacher 1992). Here, we use the general expression from L.W. Botsford and T.C. Wainwright (unpublished data):

$$\begin{aligned}(1)\qquad \mathrm{var}[R_{xy}(k)] = {} & \frac{1}{N-|k|}\sum_{v=1-N+|k|}^{N-|k|-1}\left[1-\frac{|v|}{N-|k|}\right] \\ & \times [\rho_{xx}(v)\rho_{yy}(v)+\rho_{xy}(v+k)\rho_{xy}(k-v)] \\ & -\frac{2\rho_{xy}(k)}{N}\sum_{v=1-N+|k|}^{N-1}\left[1-\frac{m}{N-|k|}\right] \\ & \times [\rho_{xy}(v)\rho_{yy}(v-k)+\rho_{xx}(v)\rho_{xy}(k-v)] \\ & +\frac{\rho_{xy}^2(k)}{2N}\sum_{v=1-N}^{N-1}\left[1-\frac{|v|}{N}\right][\rho_{yy}^2(v)+2\rho_{xy}^2+\rho_{xx}^2]\end{aligned}$$

where

$$m=\begin{cases}-j & j<0\\ j-|k| & j>|k|\\ 0 & 0\le j\le|k|\end{cases}$$

and where N is the length of the time series and $\rho_{xy}(\tau)$ is the true correlation between the two time series x and y at lag τ. Many of the ways of estimating this quantity from the series themselves involve substituting estimated values of correlations for the true correlations in various simplifications of this expression (Bayley and Hammersley 1946; Chelton 1983, 1984; Drinkwater and Myers 1987; Kope and Botsford 1990; Botsford and Brittnacher 1992; L.W. Botsford and T.C. Wainwright, unpublished data).

When the null hypothesis is no correlation between the series (i.e., $\rho_{xy} = 0$ at all lags), only the first term (i.e., the one involving $\rho_{xx}(v)\rho_{yy}(v)$) is nonzero (Kope and Botsford 1990). The expression

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involving only that term can be used to estimate the variance of the estimated correlation coefficient:

$$(2)\quad \mathrm{var}\,[R_{xy}(k)] = \frac{1}{N-|k|}\sum_{v=1-N+|k|}^{N-|k|-1}\left[1-\frac{|v|}{N-|k|}\right][\rho_{xx}(v)\rho_{yy}(v)]$$

Alternatively, this expression can be rearranged, using the symmetry of autocorrelations about zero and replacing $N - |k|$ with N, to express the effective number of degrees of freedom (EDF) in the time series, i.e., the effective sample size N^* is (cf. Bayley and Hammersley 1946)

$$(3)\quad \frac{1}{N^*} = \frac{1}{N} + \frac{2}{N}\sum_{j=1}^{D}\frac{N-j}{N}\rho_{xx'(j)}\rho_{yy'(j)}$$

where D is typically equal to N. Note that when there is no autocorrelation in either of the series, the EDF is the length of the series, N.

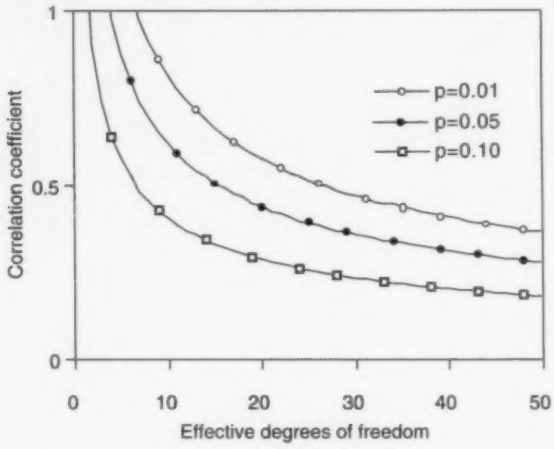
There are several important consequences of this dependence of the standard error of the estimate of a correlation coefficient on intraseries correlation. From the above expressions, it is apparent that as the degree of intraseries correlation increases, increasingly positive correlation between points at various lags will lead to a larger variance, which is equivalent to a lower number of degrees of freedom. If this dependence is ignored, i.e., if the intraseries correlation is not accounted for, the frequency at which a significant correlation would be falsely detected (i.e., a Type I error) increases as the intraseries correlation increases. For example, as time series vary from a rapidly changing series of independent points to a slowly changing series such as a moving average of four adjacent independent points, the frequency of false detection of a significant result varies from the specified value of 0.05 to a frequency of 0.26. To avoid this increase in the number of spurious correlations identified as real correlations, one can attempt to account for intraseries correlation using eq. 2 or eq. 3.

While intraseries correlation can be corrected for, it still constrains the results of correlation analysis in the sense that correlations must be stronger in order to be detected (i.e., judged significantly different from zero). The major effect of this correction on the analysis of covariability can be seen by examining the dependence of the value of correlation coefficient required to maintain a fixed error level on the amount of intraseries correlation (or equivalently, the number of independent degrees of freedom). A plot of the value of estimated correlation coefficient required to be significant at a certain probability level versus the EDF of the series (Fig. 2) shows that as the EDF decreases, the value of correlation required for a significant result increases substantially.

Because the correction that must be made for *intraseries* correlation leads to a higher required level of *interseries* correlation, it reduces the number of true relationships that we can detect to only the strongest. The implications of the above results for the detection of relationships between time series for different populations follow from the relationship in Fig. 2. To correct for the intraseries correlation in slowly varying time series, we require a higher value of correlation for statistical significance. Thus, a certain level of actual correlation between two series (e.g., 0.5) would be detectable (i.e., could be identified as significant) in rapidly varying time series (e.g., with 16 degrees of freedom or more for 0.5) but not in slowly varying series with fewer degrees of freedom.

For very slowly varying time series, it is extremely unlikely that real covariability between series will be detected. For example, the number of degrees of freedom in linear trends is approximately 2 (i.e., the number of parameters required to express a straight line); hence, covariability between two time trends would require an estimated correlation coefficient near 1.0, which is very unlikely given the amount of measurement error and other confounding noise typically associated with these series. For this reason, variability on such slow time scales is virtually undetectable. Linear trends reflect the slowest time scale, but similar, lesser effects would occur as the data varied more rapidly. For example, series dominated by half a period of a cyclic fluctuation would have only a few more degrees of freedom than a linear trend, and so on. Covariability in time series involving linear trends or up to one period of a cyclic fluctuation would be virtually undetectable, while covariability involving only a few cycles would be detected only if it were very strong.

Fig. 2. Threshold value that a correlation coefficient must have to be significant at each of three levels for various EDFs in the time series.



In spite of the fact that they are not likely to contribute a significant result, low-frequency signals such as linear trends can dominate computed correlations between two time series. This can obscure relationships on shorter time scales. For example, if there were a true inverse or negative relationship between two populations on rapidly varying time scales, yet both were declining slowly for other reasons, the computed correlation between them could be positive. The problem with this result is that the computed correlation is due to variability on an essentially undetectable time scale (i.e., a linear trend), while the true correlation remains undetected. To prevent variability on undetectable time scales from occluding variability on detectable time scales, linear trends are frequently removed from time series before correlations are computed (e.g., Kope and Botsford 1990).

Another means of removing low-frequency variability from time series is first-differencing. First-differencing removes low frequencies by effectively high-pass filtering the series; hence, it increases the number of degrees of freedom (e.g., Thompson and Page 1989). The problem with this approach is that the consequent computation of correlations between series then is focused on covariability of the first-differences of the two variables, when first-differences may not be biologically or physically meaningful. For example, the first-difference of a time series of population abundance is a confounded combination of mortality and recruitment. For this reason, we do not employ first-differencing here.

Columbia River data

We analyzed data for spring–summer yearling migrant (stream-type) chinook salmon from the following areas (Fig. 1): John Day River basin (Middle Fork, North Fork/Granite, and Upper Mainstem); Wind and Klickitat above Bonneville Dam but below other impoundments; Deschutes/Warm Springs above two impoundments; Entiat, Wenatchee, and Methow subbasin in the mid-

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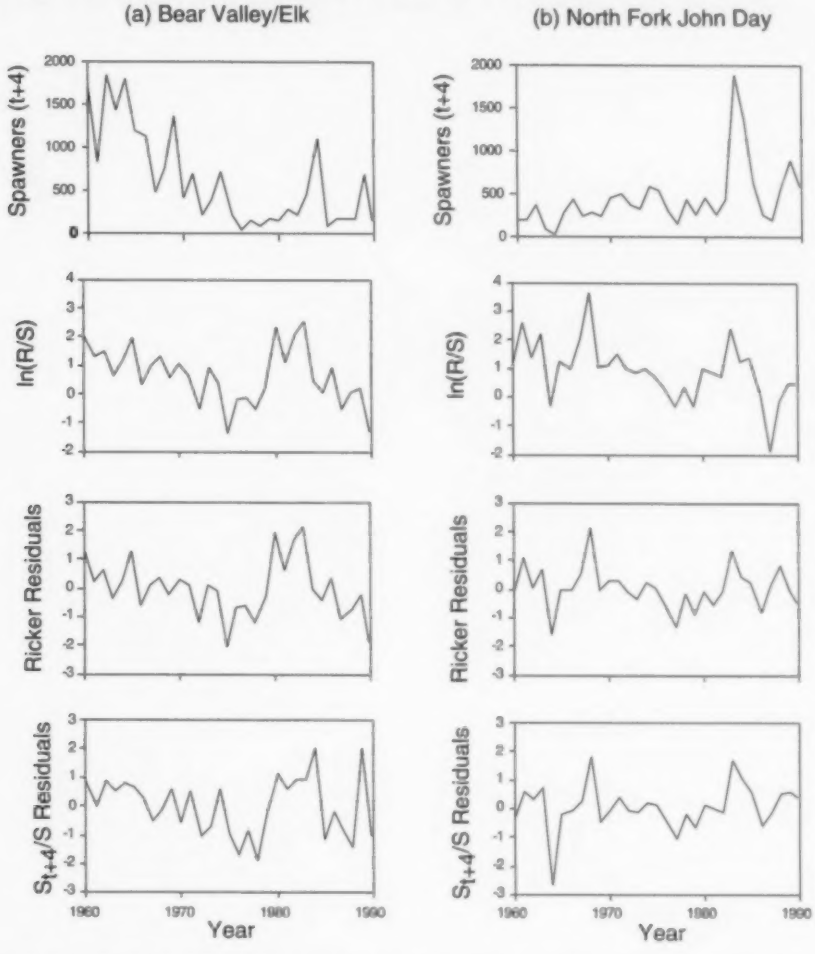
Fig. 3. Examples of different data types from an (*a*) upriver index stream and a (*b*) lower river index stream.



Columbia River; Bear Valley/Elk Creek, Marsh Creek, and Sulphur Creek in the Middle Fork of the Salmon River; Poverty Flat and Johnson Creek summer chinook almon in the South Fork of the Salmon River; Minam River spring chinook; and Imnaha River spring–summer chinook. We grouped the seven Snake River stocks (Middle Fork Salmon, Poverty Flat/Johnson, Minam, and Imnaha) into a single subbasin. We treated the mid-Columbia and the John Day streams each as separate subbasins and grouped Deschutes/Warm Springs, Wind, and Klickitat together as "lower river stocks."

We used data from spawner-at-age estimates based on redd counts and recruitment estimates based on these and subsampling for age determination by scales (R.C.P. Beamesderfer, H.A. Shaller, M.P. Zimmerman, C.E. Petrosky, O.P. Langness, and L. LaVoy, unpublished). They are based on a number of assumptions and parameters, such as peak index area redd count, total spawning area per index area, spawners per redd, and prespawning mortality, that are not known with complete certainty. From these data, we computed several different variables with different statistical and biological characteristics. The natural logarithm of the number of recruits resulting from each spawner is a commonly used population characteristic in salmon. To remove potential effects of density dependence from this relationship, we also computed the residuals about a regression based on the Ricker stock–recruitment relationship (i.e., $\ln(R/S)$ regressed on S). Lastly, we tested whether the analyses done here could be done using spawning abundance alone, without having to know the age structure from each year. We used the ratio of spawners in one year to spawners 4 years earlier. Because ages at spawning vary from approximately one third at age 4 and two thirds at age 5 on Salmon River steams to 75% age 4 and 25% age 5 in the John Day streams, this is a rough approximation of recruits per spawner.

The different types of population data (Fig. 3) have different statistical characteristics and depend on different assumptions. The most direct data are annual estimates of spawner abundance, which are based solely on the redd counts in index streams. The spawner data provide the most direct measure of population status because they do not depend on the assumptions involved in aging and run reconstruction. However, spawner abundance is inherently limited because it is the sum of several year-classes and thus contains a high degree of intraseries correlation. The residuals from detrending spawning abundance will have less intraseries correlation and hence will be a useful indicator of covariability on more detectable time scales. The natural logarithm of the number of recruits generated per spawner generating them (i.e., $\ln(R/S)$) will be even more useful for detecting covariability because it inherently involves a natural form of differencing ($\ln R - \ln S$). It will have a higher number of degrees of freedom but will depend to some de-

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Table 1. Performance of various methods of correcting xfor intraseries correlation based on simulation.

		Gaussian		Uniform	
		White series	MA series	White series	MA series
Uncorrected	Mean EDF	36	36	36	36
	Probability	0.050	0.295	0.045	0.258
$D = 30$					
Single series	Mean EDF	15.04	3.62	14.88	3.63
	Probability	<0.001	<0.001	<0.001	<0.001
Cross-correlation	Mean EDF	41.13	9.32	39.79	9.22
	Probability	0.068	0.015	0.058	0.016
$D = 10$					
Single series	Mean EDF	28.95	9.34	28.84	9.31
Cross-correlation	Probability	0.023	0.058	0.025	0.071
Single series	Mean EDF	36.58	12.16	36.38	12.05
Cross-correlation	Probability	0.057	0.039	0.047	0.047
$D = 5$					
Single series	Mean EDF	34.41	12.96	34.38	12.97
Cross-correlation	Probability	0.057	0.131	0.049	0.166
Single series	Mean EDF	36.03	18.83	35.99	18.79
Cross-correlation	Probability	0.053	0.134	0.045	0.121

Note: Mean EDF and actual probability of Type I error for a series with no intraseries correlation (i.e., white) and a four-point moving average (MA) generated from a Gaussian and a uniform distribution. D is the index of summation in eq. 3.

Table 2. Actual number of data points in spawning escapement and EDF of various data types estimated from the single series version of eq. 3.

Location	Subbasin	Years of data	Spawners	Spawners detrended	ln(R/S)	ln (R/S) detrended
Snake	Bear Valley/Elk	32	6.09	22.38	15.75	16.83
	Sulphur Creek	32	14.38	20.92	18.36	19.95
	Marsh Creek	32	8.77	20.01	15.25	16.97
	Minam	32	16.84	25.73	13.66	13.92
	Imnaha	32	17.73	24.73	16.71	17.88
	Johnson	32	8.41	25.26	22.04	21.53
	Poverty Flat	32	4.97	12.32	20.20	20.22
Mid-Columbia	Entiat	32	18.81	21.85	17.09	17.88
	Methow	31	15.35	22.15	21.22	21.13
	Wenatchee	32	24.12	23.85	7.68	23.06
John Day	Upper John Day	32	19.77	22.86	23.95	29.88
	Middle Fork John Day	32	16.99	18.18	22.43	21.99
	North Fork John Day	32	17.52	18.62	16.29	20.03
Lower River	Warm Springs	22	9.40	10.09	9.18	10.11
	Klickitat	25	19.25	19.23	12.01	12.02
	Wind	21	15.35	16.31	12.95	14.30

gree on the assumptions involved in aging and run reconstruction. The residuals from a fit to a Ricker stock–recruitment model will have a similar, high number of degrees of freedom. The ratio of spawners at one time to those A years earlier, where A is the age at which most of the spawning occurs, approximates the ratio of recruits to spawners and has the advantage of not requiring aging and run reconstructions. Here, we use the ratio of spawners at one time to spawners 4 years earlier.

The slow variability in some of these series limits the results that can be expected from analyses of covariability between the population time series. Analyses of abundance time series may show common declines in abundance, but it could be difficult to demonstrate that there is statistically significant covariability in such declines because they are on slowly varying, marginally detectable time scales. Residuals about linear trends in abundance will be good indicators of covariability on more rapidly varying, and hence more detectable, time scales. The time series reflecting survival, ln(R/S) and S_{t+4}/S_t, will vary more rapidly than abundance and hence will be useful indicators of covariability on detectable time scales. However, since a slow decline in survival is still unlikely to be detectable, a significant result will require changes on rapidly varying time scales.

Estimating EDF

To test for significant correlations between the various series, we would ideally adjust the level of correlation required for a significant result, based on computations from eq. 2 or eq. 3. The problem with this approach is that the true number of degrees of freedom is difficult to estimate. Although we can readily discuss the effects of the number of degrees of freedom in idealized time series (e.g., straight-line trends, cycles, moving averages over various periods), empirical determination of the number of degrees of

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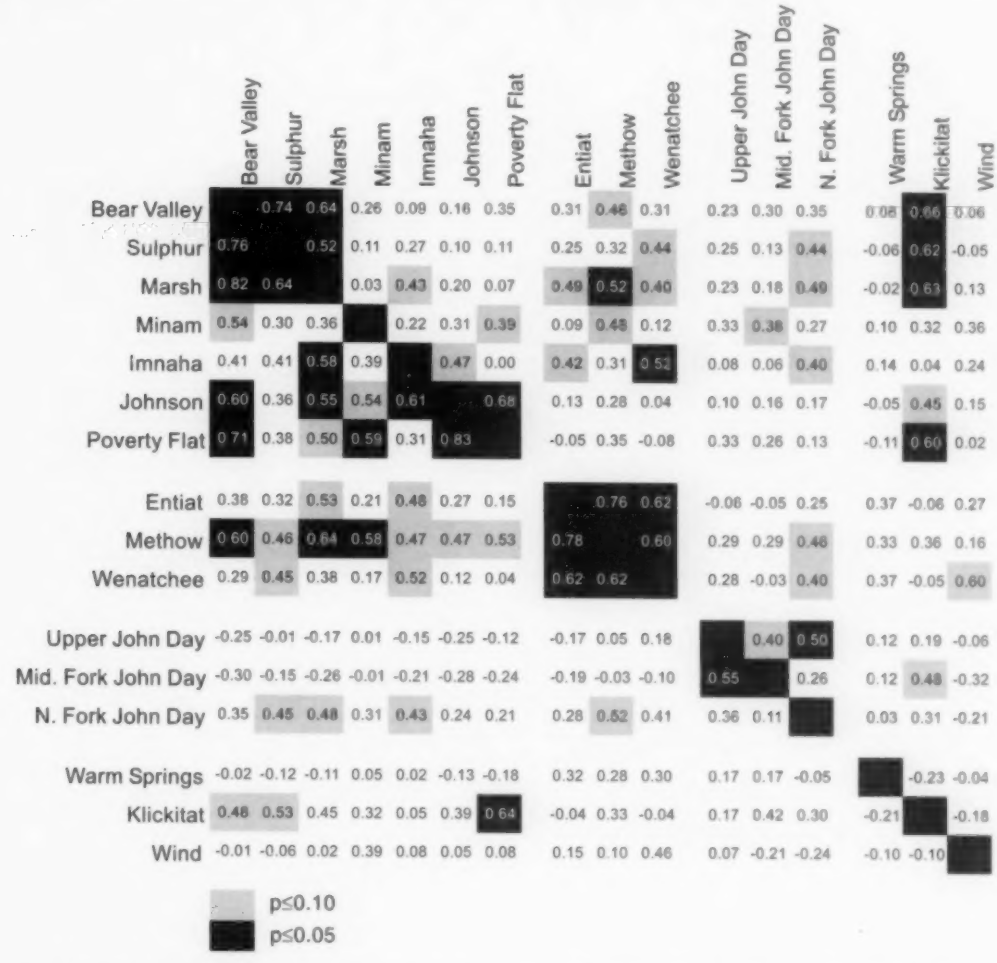
Fig. 4. Correlations between spawning escapement for specific Columbia River spring–summer chinook salmon stocks. Results for detrended series are above the diagonal, and results for raw series are below the diagonal. Levels of significance are adjusted for intraseries correlation.



freedom in a real time series is difficult. All of the various methods for estimating the standard error of computed correlations (i.e., Bayley and Hammersley 1946; Chelton 1983, 1984; Drinkwater and Myers 1987; Kope and Botsford 1990) have the fundamental limitation that they depend on the data in each series (i.e., a single realization of each process). We do not know the values of the true correlations ρ_{xx}, ρ_{yy}, and ρ_{xy} at various lags in eqs. 1 and 2 and hence must estimate them from these data. This inevitably leads to error in the estimate of the standard error of the estimate of the correlation coefficient or, equivalently, the EDF.

For the chinook salmon on the Columbia River, we take a different approach. We have 16 series of each different type of data (i.e., one per stock); hence, we can estimate the EDF using several series of each data type and then averaging them to obtain a generic number of degrees of freedom for that type. To the degree that the different series within each data type are similar in terms of intraseries correlation, this gives us a better estimate of EDF than that based on a single series. We are reducing the error caused by the fact that each series is a single realization.

There are two possible ways of determining the average EDF for a number of similar time series. One could compute the EDF of each single series and then average those. This method would use eq. 3 with ρ_{xx}^2. Alternatively, one could compute the EDF from pairs of series. This method would use eq. 3 with ρ_{xx} ρ_{yy}. This method more explicitly accounts for the fact that we are interested ultimately in computed cross-correlations.

We estimated the EDF using a modified version of eq. 3, which is less susceptible to variability in the estimates of the values of ρ_{xx} and ρ_{yy} from the limited data. Because higher values of the index of summation involve values of ρ_{xx} and ρ_{yy} at higher lags, for which estimates will be less precise because they are based on fewer data points, we reduced the upper limit of summation, D, to a number less than N.

We evaluated the relative effects of using EDF from single series versus pairs of series and the effects of various values for D (D = 5, 10, 30) by simulating the results of using each to estimate the EDF in series with no correlation (i.e., white series) and series composed of a four-point moving average. To test for an effect of the distribution of the data points on the results, we performed these simulations for data with a Gaussian distribution and a uniform distribution. We chose the limit of the summation and the type of correlation to use based on the combination that resulted in a prob-

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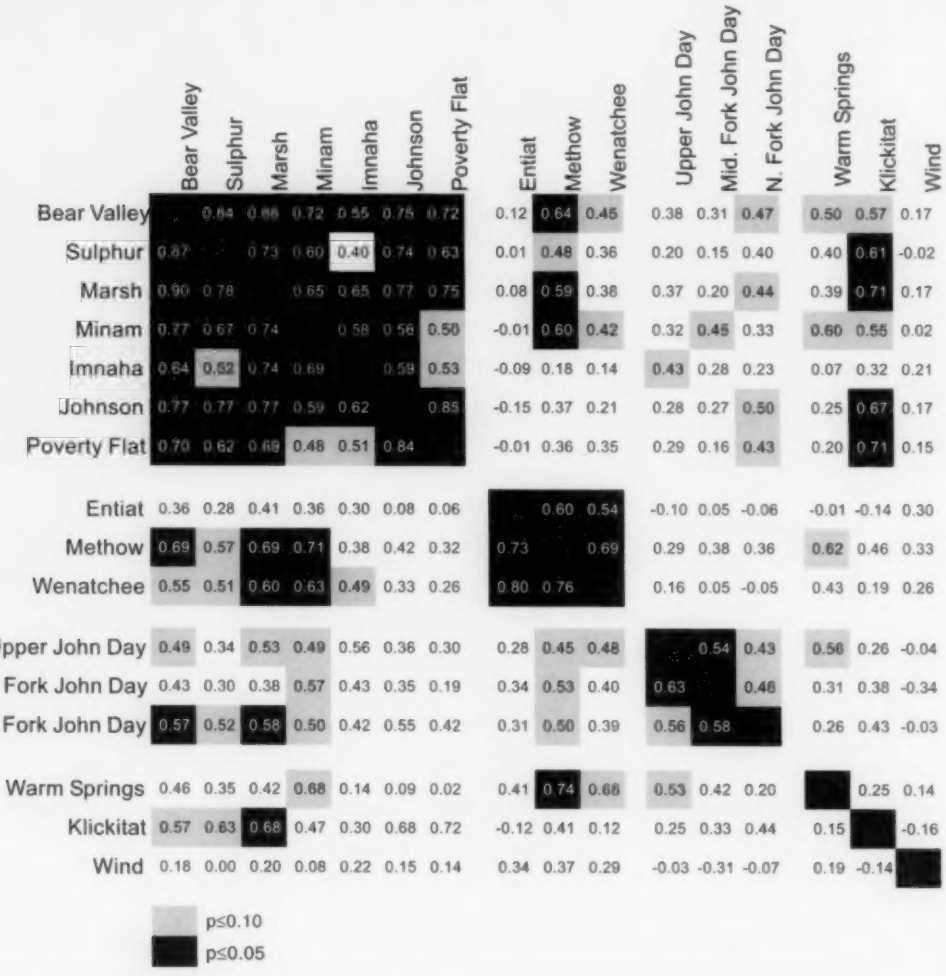
Fig. 5. Correlations between natural logarithms of recruits per spawner for specific Columbia River spring–summer chinook salmon stocks. Results for detrended series are above the diagonal, and results for raw series are below the diagonal. Levels of significance are adjusted for intraseries correlation.



ability of Type I error closest to the specified error (i.e., 0.05) for both white series and four-point moving averages.

The results of these trials indicate an index of summation of D = 10 in eq. 3 using pairs of series rather than single series gives probabilities of type I error that are most consistently close to 0.05 (Table 1). In Table 1, the effect of intraseries correlation that has to be corrected for is shown in the uncorrected case, the increase of probability of a Type I error from 0.05 for the white series to 0.295 for series that are four-point moving averages of white series. Corrections based on single series estimates overestimate the variance, leading to an underestimate of the EDF, a high significance threshold, and a low probability of Type I error. Of the paired series estimates, the variance is underestimated at D = 5 and overestimated at D = 30, with the consequent expected changes in probability of Type I error. Whether the series are Gaussian or uniformly distributed appears to make little difference for these results.

Based on this result, we computed the EDF (N^*) for each data type (spawners, ln(R/S), etc.) by calculating the variance from eq. 3 with D = 10 for each of 63 pairwise combinations (i.e., there are 63 possible pairwise correlations for the indicators for 16 stocks). We did not allow the EDF to exceed the series length N. We then used the 63 N^* values to calculate an average EDF for that data type. Because the series differed slightly in length, we had to express the degree of autocorrelation in relative terms, i.e., N^*/N. We used the ratio of the average N^* to the average uncorrected EDF. To estimate the EDF for a given pairwise correlation, we next calculate EDF(x,y) as (average N^*/average N) × (N(x,y)). This EDF(x,y) was then used to calculate the significance of each correlation.

Results

Although the simulations indicated that EDFs calculated from single series are biased low, they are our only source of information for comparison of the relative EDF for the individual streams (Table 2). Some of the Snake River streams had low EDFs, as might be expected from the dominance of a linear trend. Note the low EDF for Bear Valley/Elk Creek due to the dominant declining trend in Fig. 3. In all but two cases, detrending increased the EDFs, as would be expected.

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Fig. 6. Correlations between residuals from fits to Ricker stock–recruitment relationships for specific Columbia River spring–summer chinook salmon stocks. Results for detrended series are above the diagonal, and results for raw series are below the diagonal. Levels of significance are adjusted for intraseries correlation.

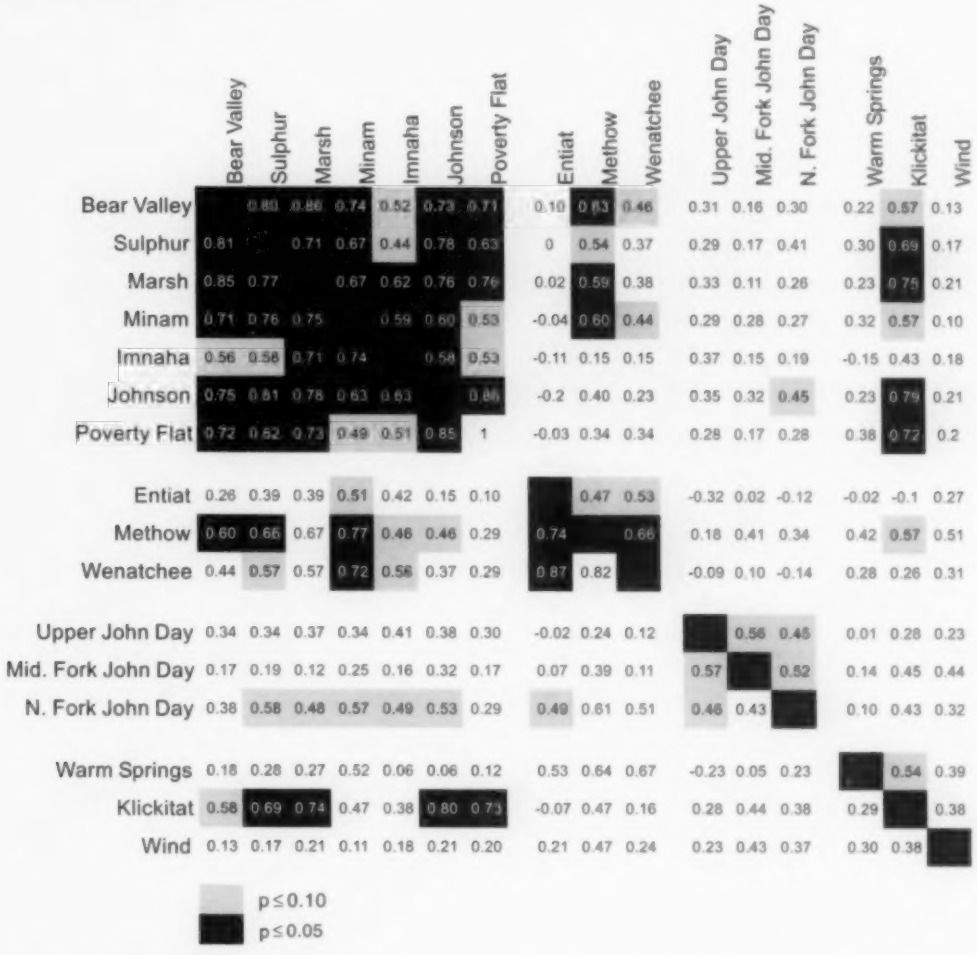

Table 3. Average EDF for eight generic types of time series from averages over all streams of estimates for cross-correlation.

Stock indicator	EDF	EDF detrended
Spawning escapement	20.4	25.5
ln(*R*/*S*)	19.0	21.6
Ricker residuals	18.5	20.5
S_{t+4}/S_t	23.4	24.8

In more than half the cases, EDF for the logarithm of recruits per spawner was greater than that for spawners. A striking exception is the Wenatchee River.

The average EDFs computed from all combinations of pairs within each series type ranged from 18.5 for Ricker regression residuals to 25.5 for detrended spawners (Table 3). In all cases, detrending increased the EDF, as expected. It is somewhat surprising that the EDF of spawning escapement is slightly larger than the EDFs of the other types of series. Since we had expected that spawning escapement would have a lower EDF, we recomputed the mean group EDF for the Snake River subbasin only to see whether the other streams had inflated that EDF. This resulted in an EDF of 13.3, which is much less than the value of 20.4 in Table 3 but slightly greater than 11.1, the average value of the single series estimates in Table 2.

We present the correlation results as a correlation matrix for each data type and both normal (lower half) and detrended (upper half) data with two significance levels, both based on the correction for intraseries correlation for that data type (Figs. 4–7). We then summarize these results on the subbasin spatial scale by the fraction of significant correlations between subbasins (Fig. 8).

The spatial pattern of covariability indicated by correlations computed from the spawning escapement data is moderately consistent covariability between stocks within the three subbasins, with much less covariability between the Snake River subbasin and the mid-Columbia (Fig. 4). The

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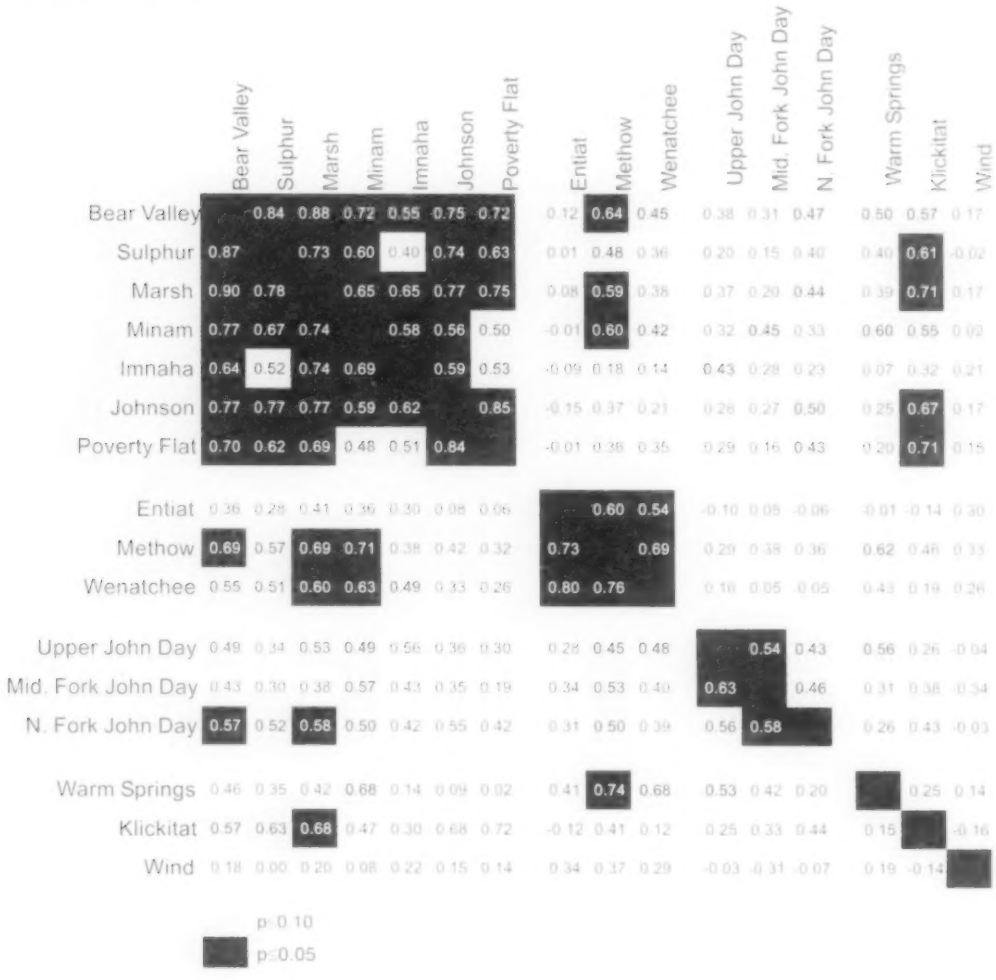

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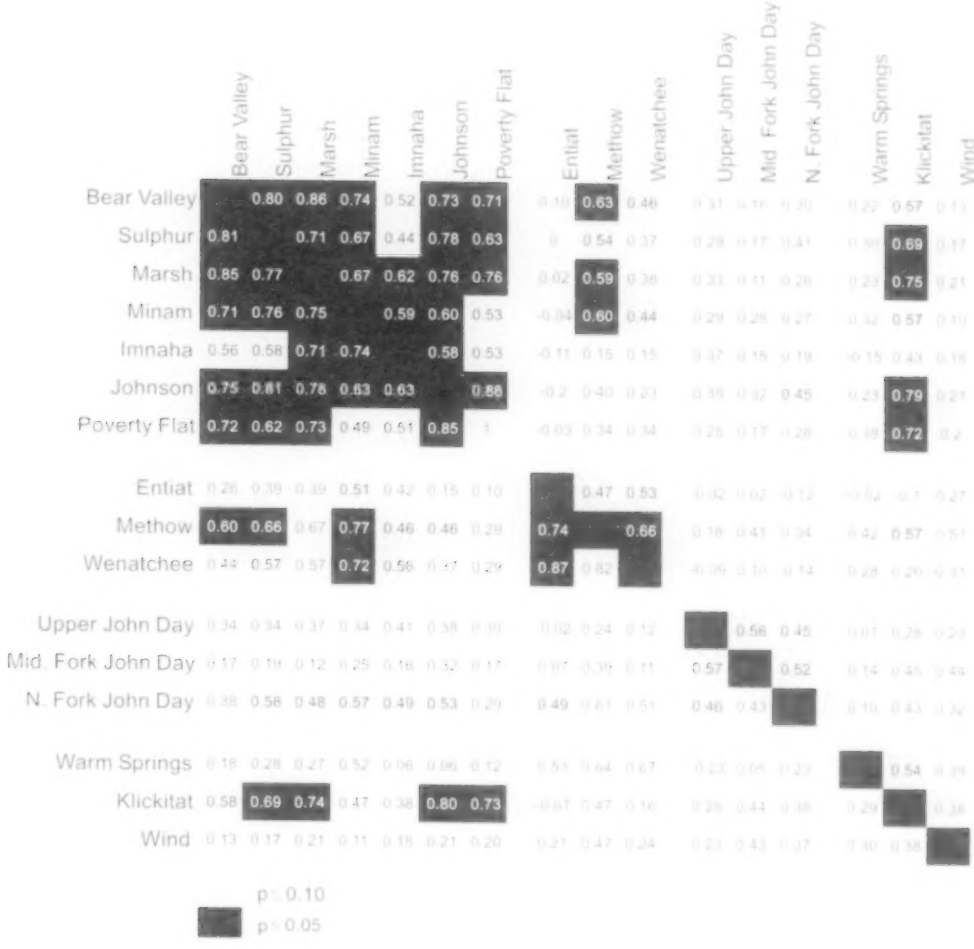

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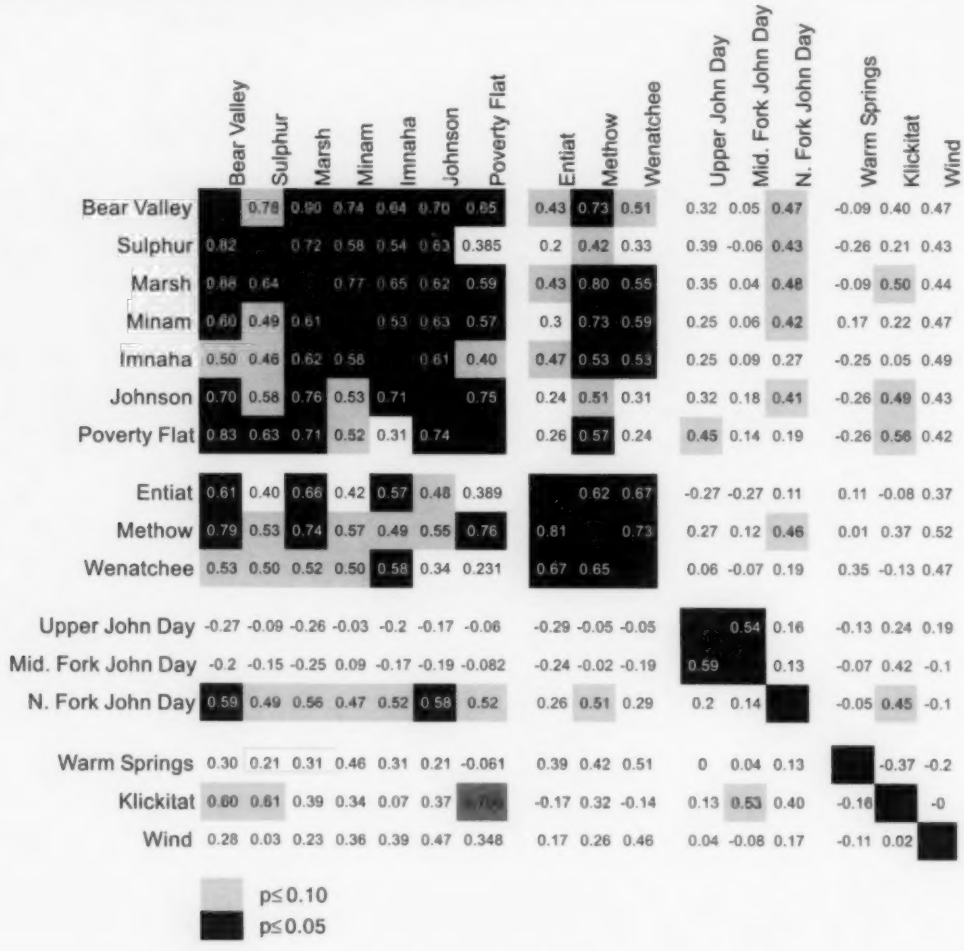
The spatial pattern of covariability indicated by correlations computed from the spawning escapement data is moderately consistent covariability between stocks within the three subbasins, with much less covariability between the Snake River subbasin and the mid-Columbia (Fig. 4). The

Fig. 7. Correlations between the ratio of spawning escapement in one year to escapement 4 years earlier for specific Columbia River spring–summer chinook salmon stocks. Results for detrended series are above the diagonal, and results for raw series are below the diagonal. Levels of significance are adjusted for intraseries correlation.



fraction of correlations that are significant within subbasins is much greater than that between subbasins (Fig. 8). Within subbasins, there are fewer significant correlations in the detrended data, indicating that part of the correlation was due to low-frequency variability. If the correlation between detrended series is less than the correlation between raw series, then the sign of the correlation in the trend is the same as the relationship at higher frequencies, positive in this case. The correlation between the Klickitat River and some of the Snake River stocks, which is stronger in the detrended data, is notable because of the large distance between the stocks (Fig. 1).

The spatial pattern in the correlations among logarithms of recruits per spawner is almost the same as that indicated by the spawner escapement data, greater covariability within the three subbasins than between them, but the pattern is clearer (Figs. 5 and 8). The strongest relationships are within subbasins, with all streams significantly correlated with all other index streams in their subbasin for all three subbasins. The correlations are uniformly higher than those from the spawner escapement data (Fig. 5) and more are significant (Fig. 8), indicating that the spatial pattern in the correlations probably results from covariability in survival rather than in abundance. Correlations in the detrended data are again generally less. The pattern indicated by the detrended data is essentially the same as for the raw data; however, the detrended correlations for the mid-Columbia subbasin are substantially lower, indicating that part of the correlations among these stocks are due to a common trend of the same sign. The significant relationships between Klickitat River and Snake River subbasin stocks are again stronger in the detrended data.

The spatial pattern in the Ricker residuals is the same as that in the logarithm of recruits per spawner (Fig. 6). For this data type, however, correlations are slightly lower, and there are fewer significant correlations between subbasins (Figs. 6 and 8). Correlations from the detrended data are generally less than the corresponding values for data without

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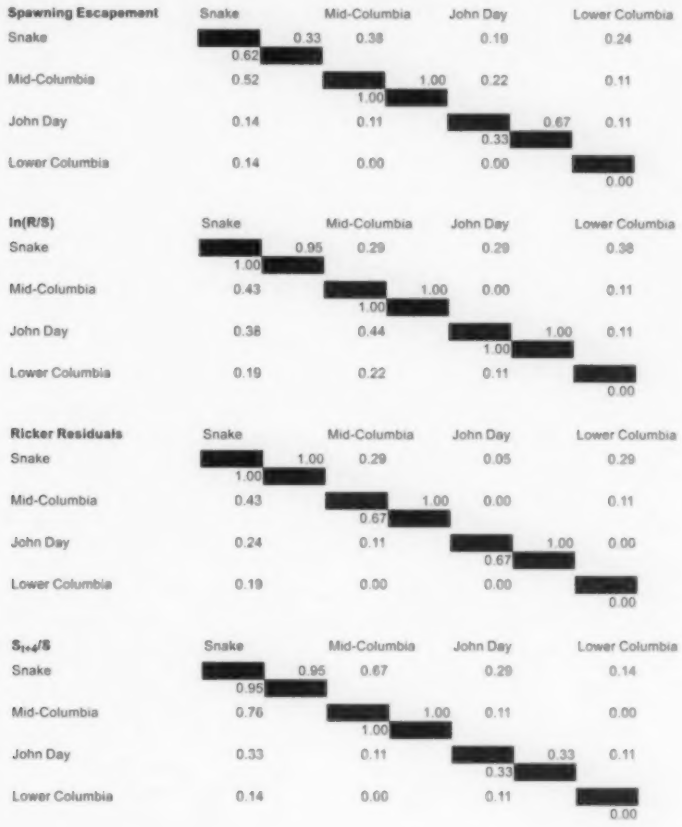
Fig. 8. Summary of correlation results: the proportion of each metric that was significant at the 0.10 level after correcting for the EDF in each generic type of series.



the trends removed, indicating that the correlations at all locations were due in part to trends. Covariability between the Klickitat River and the Snake River subbasins is present in this data type also.

The spatial pattern in the ratio of spawning escapement in one year to spawning escapement 4 years earlier differs from that of the raw spawning escapement data in that the correlations within each subbasin are stronger (Figs. 7 and 8). It reflects the same spatial pattern as the logarithm of recruits per spawner and the Ricker residuals better than the spawning escapement data, except that correlations between the Snake River subbasin and the mid-Columbia are greater. A possible drawback to its use as a replacement for recruitment data is the fact that it indicates more between-subbasin relationships than the types of data involving recruitment.

In all of these types of data, the strongest intersubbasin relationships seem to be between the Snake River subbasin and the mid-Columbia, with a lesser relationship with the John Day. The mid-Columbia and the John Day are related to each other but not as strongly.

Discussion

The dominant characteristic of the spatial pattern among these Columbia River spring–summer chinook salmon stocks is covariability among survival indicators within the three subbasins. This pattern is strongest in the three types of data directly reflecting survival rather than abundance. The fact that this appears in data reflecting survival rather than abundance is not surprising. Survival varies from year to year, while abundance is the cumulation of many random past survivals. Covariability between subbasins is only moderate but is strongest between the Snake River subbasin and the mid-Columbia. These indications of intersubbasin relationships are weaker in the higher frequency series (i.e., the series with trends removed and the Ricker residuals). The large distance between the Klickitat River and the Snake River subbasin suggests that the unusual covariability between these stocks is spurious.

This pattern of spatial covariability implies either a biological link between populations that is stronger within basins or common environmental influences within basins. The only known biological link is straying between spawning streams, but magnitudes of straying for Pacific salmon are far too low (on the order of 10% or less) to account for the correlations seen here. Common environmental influences could occur in either the freshwater or the marine phase of salmon life history. In the former, possibilities are either differing amounts of rainfall, different flow rates between basins, or different water management in each subbasin.

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Subbasin differences in the marine phase would require that stocks from the different subbasins migrate to different locations in the ocean.

This analysis demonstrates both the value of accounting for intraseries correlation and a new means of doing so when several similar series are involved. The level at which a correlation coefficient would have been termed significant for most of these series if intraseries correlation were not accounted for is roughly 0.3 (N = 30) (Fig. 2). With this as a criterion, one can readily see from Figs. 4–7 that many more correlations would have been identified as significant, thus blurring the observed spatial patterns.

A common problem in attempting to account for intraseries correlation by adjusting the level of significance as in eq. 2 (or equivalently by reducing the number of degrees of freedom, as in eq. 3) is that the estimates of ρ_{xx} and ρ_{yy} that we must use in these adjustments are imprecise and lead to random variability in the adjustment. Here, we demonstrate a method of reducing that variability by combining the estimates of variance from several similar series, thus "averaging" out the effects of variability in estimates from each series. That adjustment assumes that similar data from similar stocks, e.g., spawner abundance data from chinook salmon stocks with similar age structure, have similar intraseries correlation. While this method is effective, our results also show that if the series are not similar, important differences in EDFs may be missed. For example, spawner data from the declining Snake River stocks had a low EDF when only the eight of them were considered, yet when combined with the other stocks, the EDF was 20.1 (Table 3).

Since we began this study, a comparison of various ways of removing the effects of intraseries correlation in correlation analysis has appeared that concludes that a formulation similar to eqs. 2 and 3 is advisable. Pyper and Peterman (1998) empirically test several methods of estimating EDF similar to eqs. 2 and 3. On the basis of slight differences in simulated error probabilities, they used a form of eqs. 2 and 3 without the triangular weighting function that arises in eq. 1. We prefer to keep that derived aspect of the formulation because it naturally gives greater weight to estimates of ρ_{xx} and ρ_{yy} at low lags, which will be more precise. In their formulation, a value of D in eq. 3 of $N/5$ gave the best results for the conditions simulated, while we chose a value of $N/3$ (i.e., D = 10).

The EDFs from the different data types demonstrate the effects of intraseries correlation and the usefulness of different data types in correlation analysis. EDFs from single series (Table 2) are low in series with the strongest declining trends, the Snake River subbasin, and detrending to remove low-frequency components seemed to have the greatest effect in these autocorrelated series. Series of $\ln(R/S)$ have greater EDFs in raw form; hence, their EDFs do not increase as much with detrending because the raw series have been "differenced" in a natural way that removes intraseries correlation without removing biologically important variability.

These results suggest that for this kind of analysis, the preferred type of data would be the logarithm of recruits per spawner or the Ricker residuals. Correlations were higher than in the abundance data, indicating that the covariability is due to survival rather than to abundance. For these data, removing the effects of density dependence (i.e., the Ricker residuals) had little effect on the spatial pattern of correlation, indicating that whatever density dependence is present is neither responsible for nor occluding the covariability among the stocks.

The similarity between the spatial pattern of covariability indicated by the analysis of spawners in one year divided by spawners 4 years earlier is encouraging (also note the similarity in time series in Fig. 3). Here, the fact that we detect the same pattern using a ratio of spawners as we do by including recruitment supports our assumption that the pattern of covariability was not introduced into the estimates of recruitment by (R.C.P. Beamesderfer, H.A. Shaller, M.P. Zimmerman, C.E. Petrosky, O.P. Langness, and L. LaVoy, unpublished). While spawner and recruit data are the most desirable for this type of analysis (also see Peterman et al. 1998), they require knowing the age structure of spawners. Since there are spawner abundance data available for many more stocks for which recruitment data (or aging) are not available, analyses similar to this one can be performed over a wider scale. This ratio successfully detects covariability of survival reflected in the logarithm of recruits per spawner and Ricker residuals but appears to be less selective than those data types. This transformation of spawner data will work best for stocks with a dominant single age of reproduction.

Although we have carefully accounted for EDF so that we could weigh the relative importance of correlations with different types of data, the overall number of degrees of freedom is much lower than the figures used due to a complete lack of accounting for multiplicity of tests. The results described here should not be considered a formal hypothesis test in the sense that we have statistically proven that any of the results did not arise from chance alone. We consciously sacrificed the ability to do formal hypothesis testing for the sake of searching for patterns among as wide a suite of geographical locations and data types as possible. The next step would be testing these relationships through other means.

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References

Bayley, G.V., and Hammersley, J.M. 1946. The effective number of independent observations in an autocorrelated series. J. R. Stat. Soc. Ser. B (Methodol.), **8**: 184–197.

Botsford, L.W., and Brittnacher, J.G. 1992. Detection of environmental influences on wildlife: California quail as an example. *In* Wildlife 2001: populations. *Edited by* D.R. McCulloch and R.H. Barrett. Elsevier Applied Science, London. pp. 158–169.

Campbell, A., and Mohn, R.K. 1983. Definition of American lobster stocks for the Canadian Maritimes by analysis of fishery landing trends. Trans. Am. Fish. Soc. **112**: 744–759.

Chelton, D.B. 1983. Effects of sampling errors in statistical estimation. Deep-Sea Res. **30**: 1083–1103.

Chelton, D.B. 1984. Commentary: short-term climatic variability in the Northeast Pacific Ocean. *In* Aspects of climate variability

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in the Pacific and the Western Americas. *Edited by* W. Pearcy. Oregon State University Press, Corvallis, Oreg. pp. 87–99.

Cohen, E.B., Mountain, D.G., and O'Boyle, R. 1991. Local-scale versus large-scale factors affecting recruitment. Can. J. Fish. Aquat. Sci. **48**: 1003–1006.

Drinkwater, K.F., and Myers, R.A. 1987. Testing predictions of marine fish and shellfish landings from environmental variables. Can. J. Fish. Aquat. Sci. **44**: 1568–1573.

Elner, R.W., and Campbell, A. 1991. Spatial and temporal patterns in recruitment for American lobster, *Homarus americanus*, in the northeastern Atlantic. Mem. Queensl. Mus. **31**: 349–363.

Kope, R.G., and Botsford, L.W. 1990. Determination of factors affecting recruitment of chinook salmon *Oncorhynchus tshawytscha* in central California. Fish. Bull. U.S. **88**: 257–269.

Koslow, A.J., Thompson, K.R., and Silvert, W. 1987. Recruitment to northwest Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) stocks: influence of stock size and climate. Can. J. Fish. Aquat. Sci. **44**: 26–39.

Peterman, R.M., Pyper, B.J., Lapointe, M.F., Adkison, M.D., and Walters, C.J. 1998. Patterns of covariation in survival rates of British Columbian and Alaskan sockeye salmon (*Oncorhynchus nerka*) stocks. Can. J. Fish. Aquat. Sci. **55**: 2503–2517.

Pyper, B.J., and Peterman, R.M. 1998. Comparison of methods to account for autocorrelation in correlation analyses of fish data. Can. J. Fish. Aquat. Sci. **55**: 2127–2140.

Schaller, H.A., Petrosky, C.E., and Langness, O.P. 1999. Contrasting patterns of productivity and survival rates for stream-type chinook salmon (*Oncorhynchus tshawytscha*) populations of the Snake and Columbia rivers. Can. J. Fish. Aquat. Sci. **56**: 1031–1045.

Thompson, K.R., and Page, F.H. 1989. Detecting synchrony of recruitment using short, autocorrelated time series. Can. J. Fish. Aquat. Sci. **46**: 1831–1838.

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Social dominance, growth, and habitat use of age-0 steelhead (*Oncorhynchus mykiss*) grown in enriched and conventional hatchery rearing environments

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Abstract: This study investigated whether culturing age-0 steelhead (*Oncorhynchus mykiss*) in habitat-enriched rearing tanks, containing a combination of in-water structure, underwater feeders, and overhead cover, affected competitive ability and habitat use compared with juveniles cultured in more conventional vessels. In laboratory tests, steelhead juveniles grown in the enriched tanks socially dominated size-matched competitors grown in conventional tanks. When both treatments were introduced into separate sections of a quasi-natural stream, no differences in growth were found between them. However, when intermixed, fish reared in the enriched tanks grew at a higher rate than conventionally reared competitors, suggesting greater competitive ability of juveniles grown in the enriched tanks. Visual isolation and defensible food resources in combination in the enriched tanks were considered as the primary factors causing the observed competitive asymmetries. Steelhead juveniles from the two rearing environments exhibited very similar use of woody structure in the quasi-natural stream, both in the presence and in the absence of mutual competition. Rearing steelhead in more naturalistic environments could result in hatchery fish that behave and integrate into the postrelease (natural) environment in a manner more similar to wild fish.

Résumé : Dans cette étude, on a examiné si l'élevage de saumons arc-en-ciel (*Oncorhynchus mykiss*) d'âge 0 dans des bassins à habitat enrichi, contenant des structures et des nourrisseurs sous-marins ainsi qu'un couvert en surface, modifiait la compétitivité et l'utilisation de l'habitat, comparativement à l'élevage de juvéniles dans des bassins plus classiques. Lors de tests en laboratoire, on a observé que les saumons arc-en-ciel juvéniles élevés dans les bassins enrichis dominaient socialement leurs compétiteurs de même taille élevés dans des bassins classiques. Quand les poissons exposés à ces deux milieux d'élevage ont été introduits séparément dans deux sections distinctes d'un cours d'eau quasi naturel, on n'a observé aucune différence dans la croissance des poissons des deux groupes. Cependant, quand les deux groupes de poissons y ont été mélangés, les poissons élevés dans les bassins enrichis ont montré une croissance supérieure à celle des poissons élevés dans les bassins classiques, ce qui laisse penser que les premiers étaient plus compétitifs. On présume que les possibilités d'isolement visuel combinées à la présence de ressources alimentaires défendables dans les bassins enrichis sont les principaux facteurs responsables de cette différence de compétitivité. Les saumons arc-en-ciel juvéniles des deux types de bassins d'élevage utilisaient de façon très similaire les structures ligneuses dans le cours d'eau quasi naturel, tant en présence qu'en absence de compétition entre poissons des deux groupes. L'élevage des saumons arc-en-ciel dans des conditions plus naturelles pourrait donner des poissons d'écloserie plus semblables aux poissons sauvages au chapitre du comportement et de l'intégration au milieu naturel après leur lâcher.

[Traduit par la Rédaction]

Introduction

Recent "threatened" and "endangered" listings of Pacific salmon (*Oncorhynchus* spp.) by the National Marine Fisheries Service (NMFS) under the U.S. Endangered Species Act have prompted recovery programs that include captive propagation as a major enhancement component (e.g., Flagg et al. 1995). However, considerable controversy exists on whether cultured salmonids are suitable for rebuilding wild populations (Hilborn 1992). In fact, both genetic and developmental (i.e., environmental) factors are known to cause divergence of cultured salmonids from the wild state (Maynard et al. 1995; Waples 1999).

For Pacific salmon, conservation hatchery strategies that

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more closely emulate natural rearing conditions have been proposed to aid in the recovery of depleted wild populations. A fundamental assumption is that improved rearing technology will reduce environmentally induced behavioral deficiencies presently associated with cultured salmonids. Under the conservation hatchery paradigm (Flagg and Nash 1999), efforts to improve the survival of salmon smolts released from hatcheries have included structural modifications to hatchery rearing vessels intended to provide "enriched" environments more similar to those in natural streams. Such modifications have included a combination of underwater feed-delivery systems, submerged structure, overhead shade cover, and gravel substrates or some subset of these variables (Maynard et al. 1995). These enriched rearing environments have been demonstrated to improve instream survival of chinook salmon (*Oncorhynchus tshawytscha*) smolts during seaward migrations in some (Maynard et al. 1996) but not all studies (Berejikian et al. 1999). The concept aims in part to promote the development of more natural behavior patterns (Maynard et al. 1995), but thus far, no behavioral differences between juvenile salmonids grown in "conventional" and enriched habitats have been identified.

Fitness-related behavioral attributes of salmonids can be influenced by many environmental factors. In particular, manipulation of environmental factors during culture such as fish density (Fenderson et al. 1968; Berejikian et al. 1996), food ration (Symons 1968; Ryer and Olla 1991; Berejikian et al. 1996), and method of food introduction (e.g., localized versus dispersed: Ryer and Olla 1995) influence social behavior. Differences also exist between juvenile salmonids grown in hatcheries and streams for attributes such as agonistic behavior (Berejikian et al. 1996) and microhabitat use (Dickson and MacCrimmon 1982). These differences illustrate the need to modify culture techniques to minimize developmental and possibly evolutionary divergence between artificially propagated and wild populations.

Steelhead (*Oncorhynchus mykiss*) along the west coast of North America reside in streams for 1–4 years (typically 2 or 3 years) before migrating to sea. Hatchery programs designed to augment harvest rear steelhead to the smolt stage in 1 year and release them. Programs releasing steelhead at earlier life history stages (e.g., age 0) may produce fewer smolts than programs that rear fish to the smolt stage prior to release. However, releasing steelhead earlier in their life history might minimize the developmental (e.g., behavioral) impacts of captive culture, which can occur rather quickly (Berejikian et al. 1996) and may negatively affect breeding success (Atlantic salmon (*Salmo salar*): Fleming et al. 1997). In fact, conservation hatcheries designed to reestablish self-sustaining populations of other Pacific salmon species use a variety of reintroduction strategies that involve releases at various life history stages (e.g., sockeye salmon (*Oncorhynchus nerka*): Flagg et al. 1995). Recent listings of some steelhead populations under the U.S. Endangered Species Act present the likelihood that various release strategies, including release as age-0 juveniles, will also be implemented for steelhead reared in conservation hatcheries.

The main objective of the present study was to examine several important behavioral parameters to gain a better understanding of how juvenile steelhead grown in conventional and enriched environments might assimilate into natural (i.e., stream) environments after release. Specifically, we investigated whether steelhead juveniles grown in enriched rearing tanks containing a combination of in-water structure, underwater feeders, and overhead cover would differ from juveniles grown in conventional vessels with respect to (*i*) social dominance, (*ii*) growth in the presence and absence of mutual competition, and (*iii*) use of woody debris structure. In the present study, the performance of steelhead juveniles in laboratory dominance trials was compared with evaluations of growth under competitive conditions in a quasi-natural stream channel.

Methods and materials

Study population, rearing treatments, and tagging

Eyed embryos were obtained from artificially spawned steelhead from the Skookumchuck River, Mason County, Wash. This hatchery population was derived from the local wild population and spawning protocols have continued to incorporate naturally produced (i.e., wild) steelhead into the spawning broodstock each year. Eyed embryos (n = 4800) were sampled from the spawning of 12 females with 12 males. The eyed embryos were transported to the NMFS facility at the University of Washington's Big Beef Creek Research Station for final incubation in constant 10°C well water. The entire lot of embryos was mixed thoroughly to minimize possible kinship effects on social interactions.

Six hundred and fifty fish were stocked into each of six 1.8-m-diameter circular tanks (water volume = 1520 L, flow = 28 L·min^{-1}) on 24 May 1998. Three enriched tanks contained the tops of two commercially grown Douglas-fir (*Pseudotsuga menziesii*) trees that were submerged to provide in-water structure. The total volume encompassed by the outline of the trees was approximately 315 L (about 21% of the water volume in the tanks). A double layer of brown and green camouflage netting hung on a circular PVC frame provided approximately 60% overhead shade cover. Each tank was outfitted with an underwater feed-delivery system, which delivered food at the midwater depth at two locations on opposite sides of the tanks (Fig. 1). Three conventional tanks contained no overhead cover or in-water structure (other than a centre standpipe) and received water at 28 L·min^{-1} from above the water surface. Fish in the conventional tanks were hand-fed by scattering the food across the surface of the water. Fish in all tanks were fed with equal frequency, which was gradually decreased from about 12 times per day at the beginning of rearing to four times per day at the time the fish were sampled for the experiments.

All of the fish in each tank were marked from 6 to 9 July 1998 with a visible latex tag (Pow'rject System, Newwest Technologies, Santa Rosa, Calif.; reference to trade names does not imply endorsement by NMFS) injected into the anal fin tissue. Fish in each tank were marked with a different color. Those in the three enriched tanks received blue, red, or white tags, and fish in the conventional tanks received purple, orange, or green tags. The resulting tag was visible in the tissue between several rays of the anal fin.

Dominance and aggressive behavior

Flume apparatus

Comparisons of dominance status and aggressive behavior were conducted in two 10-m-long by 1.5-m-wide flumes located at the NMFS Manchester Marine Research Station. Each flume was divided longitudinally with a solid divider, and screens were placed perpendicular to the flow to produce twenty-two 0.75-m-long by 0.75-m-wide sections in each flume (not all of the sections in each flume were used). The substrate of each section consisted of a 5-

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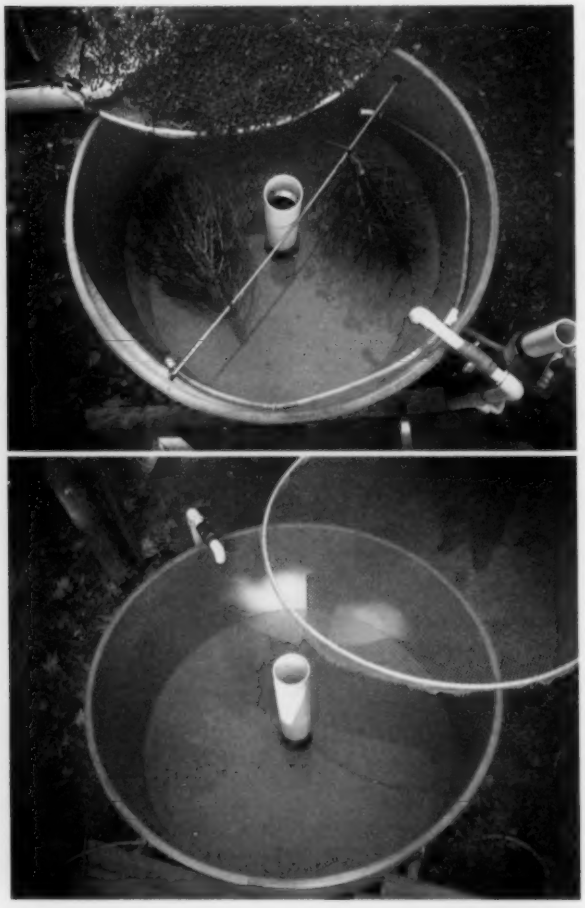
Fig. 1. Top view of the (A) enriched and (B) conventional rearing tanks. Structure in the enriched tanks consisted of denuded conifers. The enriched tanks were also covered with camouflage netting (brown and green), which provided approximately 60% shade cover.



cm-thick layer of 1.0- to 1.5-cm-diameter gravel. Each flume received 12°C well water at 30 L·min^{-1}, which was recirculated in each flume at a flow of approximately 1700 L·min^{-1}. Water depth was maintained at 24 cm. Light was provided by a solid bank of wide-spectrum fluorescent lights on a simulated photoperiod of 16 h light : 8 h dark. The side walls of the flumes consisted of double-paned glass, which allowed complete viewing of all fish in each cell. Live *Daphnia* were continuously introduced into the head box of each flume by peristaltic pumps at a rate of approximately 40 g wet weight·day^{-1} (approximately 630 individuals·h^{-1}). The *Daphnia* entered each section through the upstream screen such that, within each cell, fish in the upstream-most position had first access to the food.

Protocols

A sample of fish from each of the six tanks was placed into one of six transport containers (50 L), each supplied with oxygen. The six containers were transported 40 km to the Manchester facility. Each dominance trial included one fish from each of the six rearing tanks. The weight of fish used in the experiment ranged between 1.72 and 2.90 g. Fish were matched within 8.0% body weight within each trial, and any weight differences between tank representatives within a replicate cell were most likely randomized and therefore nondirectional with respect to treatment.

Approximately 20 h after introduction, each group of fish was observed for 3 min to make a preliminary assessment of dominance. Dominant fish were those that held a feeding station in the center third of the upstream half of the cell, moved freely about the cell, and demonstrated aggression towards other fish without themselves being chased or attacked. Dominant fish also had a uniform light background body coloration with prominent parr marks (Keenleyside and Yamamoto 1962; Abbott et al. 1985; Berejikian et al. 1996). The apparent dominant fish was then observed for 7 min to quantify the frequency of attacks (including nips, charges, and chases) and lateral displays that it delivered. Definitions of these behaviors followed Holtby et al. (1993). A fish was confirmed to be dominant only if it (*i*) maintained its feeding station until the end of the observation, (*ii*) delivered more attacks than it received, (*iii*) never exhibited submissive behavior (Keenleyside and Yamamoto 1962), and (*iv*) never retreated when attacked or approached by other fish. The position of the dominant fish was determined again about 1.5 h later to confirm that it remained in the upstream, center position. At the end of the day, each dominant fish was removed and assigned a rank of 6. The observation procedure was repeated on consecutive days. The dominant fish in each cell on the second day was given a rank of 5, and so on, until only one fish remained. The ranks were then summed for each treatment within a cell. The summed ranks for each treatment within a cell could have ranged from 15 (i.e., sum of ranks 6, 5, and 4) to 6 (sum of ranks 3, 2, and 1). A Wilcoxon paired ranks test was used to compare differences in the summed ranks. Twenty-four trials were conducted between 28 July and 1 August 1998, and 20 more trials were conducted between 4 and 8 August 1998 (n = 44).

Another experiment was conducted to determine whether the six tag colors used in the previous experiment affected dominance in a commonly reared group of steelhead juveniles. The fish were grouped by weight category (<7.5% maximum difference). Six fish within each weight category were each marked with a different color and simultaneously placed into a common section of the flume. A total of 14 trials were conducted in the same manner as the previous experiment. The effect of color on dominance rank was analyzed by a single classification Kruskall–Wallis test (chi-square approximation).

Habitat use

The habitat use experiments were carried out in a 45-m-long by 6-m-wide outdoor stream channel (3% gradient) at the Manchester facility. Well water was supplied at 80 L·min^{-1} and was recirculated at a flow of approximately 5100 L·min^{-1}. Water temperature was maintained between 11.0 and 15.5°C over the course of the study. Sixteen replicate 5.0-m-long by 3.0-m-wide sections were created in the stream channel (Fig. 2). The substrate was composed of 3- to 5-cm-diameter gravel, which was graded to create as similar a depth and velocity profile as possible in the 16 single sections. Water depth in the 16 sections averaged (±SD) 14.8 cm (3.3) near the upstream boundary and gradually increased to 27.3 cm (1.4) at the downstream boundary of each section. Algal growth on the substrate and side walls supported abundant aquatic insect populations (family Chironomidae); no artificial feed was introduced. Chironomid larvae, pupae, and adults were continuously available to the steelhead throughout the study. The unknown biomass of insects was probably similar among sections in the recirculating stream. No piscine predators were introduced, and avian predators were excluded by a single layer of fine 2.5-cm^2 mesh bird netting placed over the entire channel.

A single sliding door (13 cm high by 61 cm long) was located in the center of each longitudinal wooden wall dividing laterally adjacent sections (Fig. 2). When the doors were open, steelhead moved

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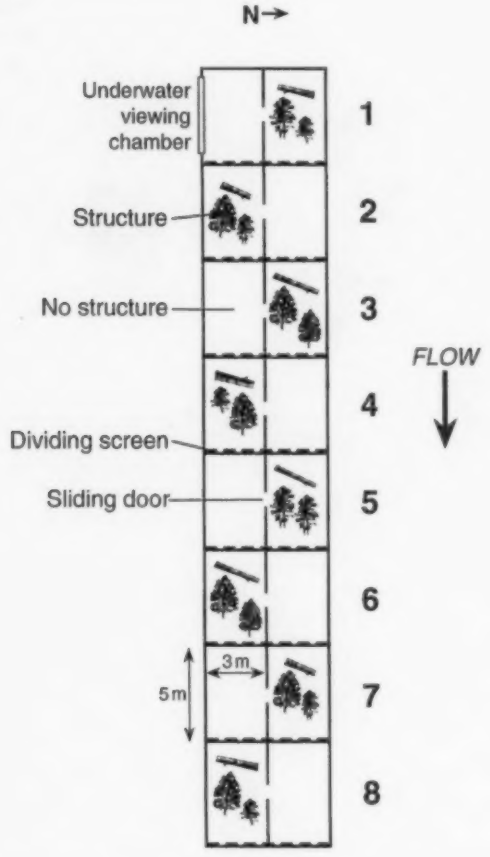
Fig. 2. Top view (not to scale) of the quasi-natural stream channel used in the habitat use and growth experiments. Submerged structure consisted of 18-cm-diameter red alder logs and denuded conifers.



freely between laterally adjacent sections (observed by underwater video). Strings were attached the doors so that they could be closed quickly (in less than 1 s) from a remote location, without human disturbance to the fish. Once the doors were closed, fish were confined to the side of the section that they inhabited just prior to door closure (Fig. 2). An underwater viewing chamber positioned alongside the entire length of one of the 16 sections allowed for general observations of steelhead behavior in both the habitat use and the growth experiments.

Two denuded, commercially farmed Douglas-fir trees (1.8 m long by 0.9 m maximum diameter) were partially submerged in half of the single sections on each side of the stream channel to provide complex submerged structure, overhead cover, and complex flow patterns. One red alder (*Alnus rubra*) log (1.2-m long by 15-cm diameter) was positioned on a diagonal across the upstream area of the structured sections. The red alder logs floated off the bottom and were held in place by strings fastened to the upstream weir boards and the center divider (Fig. 2).

The null hypothesis that prior rearing treatment had no effect on habitat use (structure versus no structure) was tested in two experiments: one in which fish from the two rearing treatments were mixed (TM) and one in which fish from the treatments were tested separately from one another (TS). For the TS experiment, 90 fish per treatment (30 from each of the three tanks within a treatment) were introduced into alternate adjoining sections on 13 July 1998. The doors on the center dividers were open so the fish could move freely from side to side. Half of the 90 fish were introduced into the side containing woody structure, and the other half were simultaneously introduced into the adjoining side containing no structure. Four paired adjoining sections received fish from the enriched tanks, and the other four pairs of adjoining sections received conventionally reared fish. Seventy-two hours after the fish were introduced, all eight doors were closed from a remote location, which confined the fish to either the structured or the nonstructured sides of the adjoining sections. At the time the doors were closed (11:00), no shadows were cast by either the walls of the channel or any other objects. All fish were then removed by a combination of seining and electroshocking, and the number of fish from each rearing tank (as identified by tag color) on each side of the adjoining sections was recorded. On 20 July 1998, the procedure was repeated, and the allocation of treatments to paired sections was reversed such that sections that received conventionally reared fish on the first set of trials received fish from the enriched tanks on the second set of trials, and vice versa. Thus, both treatments were tested in all eight adjoining sections.

The TM experiment tested the null hypothesis that prior rearing treatment had no effect on habitat use in the presence of competition from the other treatment, and it was conducted in the same manner as the TS experiment with the following differences. Fifteen fish from each of the six rearing tanks (representing both treatments) were introduced into each of the eight paired sections at 11:00 on 27 July 1998. Thus, all tanks from both treatments were simultaneously represented in all eight paired sections in one set of trials.

For both the TM and TS experiments, the proportions of fish from each treatment on the structured side of each of the eight sections were arcsine transformed to normalize the data. Proportions for each rearing tank were averaged across the eight different stream channel sections within each experiment. Comparisons between the treatments were analyzed by two-sample t tests with the rearing vessels as replicates ($n = 3$ per treatment).

Growth in a quasi-natural stream

Two experiments were conducted to test the null hypotheses that neither rearing treatment (enriched versus conventional), stream habitat type (structured versus nonstructured), nor their interaction would effect the growth rate of steelhead in a novel environment (stream channel). These hypotheses were tested with the treatments mixed (TM) and with the treatments separate (TS).

To measure individual growth of steelhead, each fish used in these experiments was injected with a passive integrative transponder (PIT) tag, which possessed a unique alphanumeric code. All fish were tagged on 10 August 1998 by anesthetizing each fish and inserting a PIT tag into the left side of the abdominal cavity with a 12-gauge needle. Each fish was then measured (fork length) to the nearest millimetre and weighed to the nearest 0.1 g. The fish were then returned to their respective rearing vessels for 2 days before being transported to Manchester and stocked into the stream channel. There were no mortalities during tagging or the 2-day recovery period. Mean fork length and weight of fish from the enriched (68.0 mm, 3.7 g) and conventional tanks (68.4 mm, 3.5 g) used in the growth experiment did not differ significantly at introduction ($P > 0.05$ for both comparisons).

For the growth experiments, the center divider doors in the stream channel were closed to create 16 separate sections (Fig. 2). The eight sections on the south side of the stream channel were used to test for differences in growth under conditions of mutual competition (TM), and the eight sections on the north side of the channel were used to test for differences in growth in the absence of mutual competition (TS). In the TM experiment, 21 fish from each treatment (seven fish per tank, 42 in total) were simultaneously stocked into each of the eight sections on the south side of

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the channel at approximately 12:00 on 14 August 1998. In the TS experiment, 42 fish from each treatment (14 fish per tank) were stocked into separate single sections on the north side of the stream channel at approximately 13:00. In this manner, sections 3, 4, 5, and 6 received fish reared in enriched tanks, and sections 1, 2, 7, and 8 received conventionally reared fish.

All fish in each section were removed 20 days (TM experiment) or 21 days (TS experiment) later, identified to rearing tank, measured, and weighed. Growth rates for weight and length (percent per day) were calculated for each fish. Within each stream channel section, individuals from a common rearing tank were averaged, and these tank means were used as the experimental unit of replication for the rearing treatment effect. For the competition experiment, a nested-factorial ANOVA was conducted to determine the effects of rearing treatment, habitat type, and their interaction on growth. The eight separate stream sections were nested within habitat type (four sections per habitat type) (Fig. 2). For the TS experiment, insufficient degrees of freedom were available to nest stream sections within habitat type, so the data were analyzed using a two-factor ANOVA with rearing treatment and habitat type as the main effects.

Results

Dominance and aggressive behavior

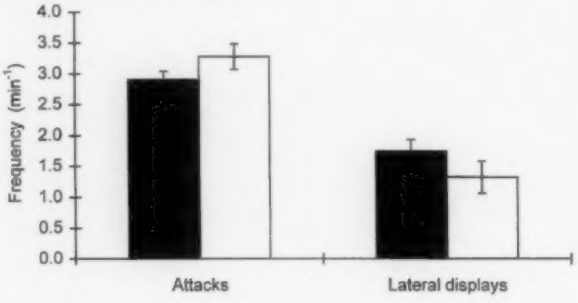
A dominant fish was clearly identified, based on the pre-established criteria, on each day for each of the 44 trials. Each dominant fish defended a feeding territory in the upstream, center area of its section. Subordinates were arrayed primarily downstream of the dominant fish with a few occasionally positioned along the lateral margins of the cell even with, or upstream from, the dominant fish. Steelhead reared in the enriched vessels had a significantly higher dominance rank (mean rank = 12.1) than those grown in the conventional tanks (mean rank = 8.9) (Wilcoxon matched pairs test, $t = 125$, $P < 0.01$). On day 1, dominant fish from the enriched treatment ($n = 30$) and dominant conventionally reared fish ($n = 14$) did not differ in their frequency of attacks ($t = 1.85$, 42 df, $P = 0.07$) or displays ($t = 1.25$, 42 df, $P = 0.22$) against the other five fish in the cell (Fig. 3). Aggressive behavior frequencies between dominants of the two treatments could not be compared statistically beyond day 1 because the number of fish remaining in each cell from each treatment differed (Table 1) and may have biased the results.

The six different tag colors applied to a commonly reared group of steelhead did not have a significant effect on dominance rank ($\chi^2 = 9.58$, 5 df, $P = 0.09$). Steelhead marked with orange tags had the highest mean rank (4.1) followed by purple (3.9), red (3.8), blue (3.6), green (2.9), and white (2.6).

Habitat use

In the TS experiment, fish from the enriched and conventional treatments demonstrated similar use of structured and nonstructured habitats. After 72 h in the stream channel, 51.6% of fish reared in enriched tanks and 52.0% of conventionally reared fish were recovered on the structured side of the paired sections ($t = 0.33$, 4 df, $P > 0.50$). When fish from both treatments were placed simultaneously into the same sections (TM experiment), 60.1% of fish reared in the enriched tanks and 59.2% of conventionally reared fish were recovered on the structured side of the paired sections ($t = 0.43$, 4 df, $P > 0.50$).

Fig. 3. Frequency of aggressive attacks and lateral displays by dominant steelhead from the enriched tanks (solid bars, $n = 30$) and dominant conventionally reared steelhead (open bars, $n = 14$) on day 1 of the experiment. Neither comparison was statistically significant ($P > 0.05$).



Growth in a quasi-natural stream

In the TM growth experiment, fish reared in the enriched tanks exhibited greater increases in length ($F_{1,38} = 8.23$, $P < 0.01$) and weight ($F_{1,38} = 6.22$, $P = 0.02$) than conventionally reared fish (Fig. 4). Habitat type (i.e., the presence or absence of woody debris structure) had no effect on the rate of length ($F_{1,6} = 0.076$, $P > 0.50$) or weight ($F_{1,6} = 0.43$, $P > 0.50$) growth; there was no interaction between habitat type and rearing treatment (length: $F_{1,38} = 0.27$, $P > 0.50$; weight: $F_{1,38} = 0.96$, $P = 0.33$).

In the TS experiment, the two treatments did not differ in their rate of length ($F_{1,20} = 0.6$, $P = 0.45$) or weight ($F_{1,20} = 0.09$, $P > 0.50$) growth (Fig. 4). Habitat type had no significant effect on length ($F_{1,20} = 3.97$, $P = 0.06$) or weight ($F_{1,20} = 1.93$, $P = 0.18$) growth. No interaction existed between the effects of rearing treatment and habitat type for either length ($F_{1,20} = 1.99$, $P = 0.17$), or weight ($F_{1,20} = 2.06$, $P = 0.17$) growth.

Observations made from the underwater viewing chamber, situated alongside the south side of section 1 (Fig. 2), indicated that steelhead held territories close to the substrate in the swiftest currents, primarily down the center third of the channel. In agonistic contests between territory holders, the fish stationed upstream usually defeated those positioned downstream of them. Fish moved laterally from their stations to intercept food in the water column, frequently removed food from the substrate, and fed quite rarely at the surface. The most dominant territory holders exhibited a strong permanence of station by maintaining their positions for most if not all of the 20-day experiment. They also appeared to be among the largest fish in the section by the end of the experiment. Some nonterritorial fish were located in backwater currents along the sidewall (window) of the section. These observations indicate that steelhead juveniles in the stream channel exhibited social patterns similar to those observed in natural streams (cf. Edmundson et al. 1968; Everest and Chapman 1972; Berejikian 1995*a*).

Discussion

In our laboratory flumes, steelhead juveniles grown in the enriched tanks socially dominated size-matched competitors grown in the conventional tanks. When both groups were in-

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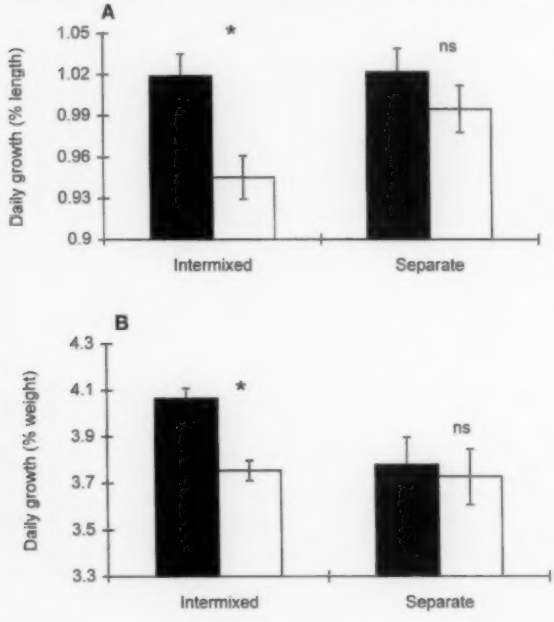
Table 1. Results of dominance trials of steelhead fry reared in enriched and conventional tanks.

	Enriched			Conventional		
Day	Number of fish	Dominant number (%)	Vacant cells	Number of fish	Dominant number (%)	Vacant cells
1	132	30 (68.2)	0	132	14 (31.8)	0
2	102	29 (65.9)	0	118	15 (34.1)	0
3	73	27 (61.4)	0	103	17 (38.6)	0
4	46	15 (41.6)	8	86	21 (58.4)	0
5	31	19 (61.3)	13	57	12 (38.7)	0

Note: Three fish from each treatment were stocked into each of 44 replicate sections (cells) of the indoor flumes. A single dominant fish on each day was removed from each section until only two fish remained on day 5. Beginning on day 2, fish from the conventional treatment outnumbered fish from the enriched treatment. The number of fish is that from each treatment that were present on each day of the experiment in all 44 trials combined. The number and percentage of dominant fish from each treatment represent only those trials (cells) in which at least one fish from each treatment remained. For example, eight trials on day 4 contained only conventionally reared fish because all three fish reared in the enriched tanks had been removed as dominant on previous days; thus, the outcomes of 36 rather than 44 trials are shown. Vacant cells are the number of cells in which no fish from that treatment remained because all three fish in the cell had been removed as dominant on previous days.

Fig. 4. Differences in mean (±1 SE) percent daily growth rates between steelhead grown in enriched (solid bars) versus conventional (open bars) rearing environments in two separate experiments: one in which the treatments were mixed and one in which the treatments were separate. Growth is presented as increases in (A) length and (B) weight. Significant ($*P < 0.05$) and nonsignificant (ns, $P > 0.05$) results are noted above the bars.



troduced into a quasi-natural stream, fish reared in the enriched tanks grew at a higher rate than conventionally reared fish under conditions of mutual competition (TM experiment). No differences in growth rate were found in the absence of mutual competition (TS experiment), indicating that growth differences in the TM experiment were caused by competitive interactions. Thus, steelhead grown in the enriched rearing environment outcompeted those grown in the conventional environments in two independent evaluations. Previous laboratory studies of juvenile salmonids, including steelhead, have found that manipulation of rearing parameters, such as fish density and food ration, affects competitive (i.e., agonistic) behavior as measured in rearing environments themselves (Atlantic salmon: Fenderson et al. 1968; Symons 1968) and after transfer to novel test environments (steelhead: Berejikian et al. 1996). The present study demonstrates that structural modifications to hatchery rearing environments, under conditions of equal ration, density, and flow, can also affect relative competitive ability in salmonids.

Steelhead juveniles grown in the enriched and conventional tanks did not differ in their preference for woody debris structure in the outdoor stream channel. Juveniles from both treatments distributed themselves about equally between sections containing structure and those containing no structure, both in the presence (TM) and the absence (TS) of mutual competition. In natural streams, age-0 steelhead use a wide range of depth and velocity, but they can be generally characterized as territorial bottom-dwellers (often associated with large rubble) utilizing shallow-water habitats. Bugert et al. (1991) found little response of age-0 steelhead to the presence of cover in a natural stream, and Hartman (1965) found them more frequently in open areas than those where log cover was present. Results of the present study suggest that innate preferences of steelhead juveniles for cover may not be much affected by providing cover during rearing. However, age-1 steelhead, which have much stronger preferences for deeper pools and cover than age-0 steelhead in natural streams (Everest and Chapman 1972; Bisson et al. 1988), may develop different affinities for cover after longer periods of rearing.

In the enriched tanks, visual isolation provided by submerged structure and defensible food resources (i.e., two underwater food inlets) probably created the conditions responsible for increasing the competitive ability of the steelhead juveniles, although other factors could have contributed. Mesick (1988) explained how the presence of complex structure in streams creates visual isolation among potential competitors and limits the total frequency of aggressive interactions. Fewer attacks from potential competi-

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tors reduces so-called "intruder pressure." Reduced intruder pressure allows more individuals to establish and defend territories (Grant and Noakes 1987) and thereby may result in more completely developed innate territorial behavior and increased competitive ability. Further support for this concept is evident from other studies (Berejikian et al. 1996; Keeley and McPhail 1998) that have demonstrated that aggressive behavior or territory size decreases when intruder pressure is high (e.g., at high densities). Under such conditions, the benefits of territorial behavior may not outweigh the costs in terms of energy expenditure (Li and Brocksen 1976) and injury (Abbott and Dill 1985) associated with high levels of agonistic activity. In addition to providing visual isolation, the submerged structure in the enriched tanks (present study) also provided reference objects, which Hartman (1963) demonstrated salmonids use to establish and defend territories. For territorial tactics to be adopted, however, individuals must receive an energetic "payoff" that outweighs the cost of territory defense.

The two underwater feeder inlets in each of the enriched tanks provided defensible food sources and therefore a benefit (increased food intake) to territorial behavior. By contrast, fish in conventional tanks received food that was scattered across the water surface and could not be monopolized. Ryer and Olla (1995) found that localizing food in rearing tanks increased the frequency of agonistic behavior in chum salmon (*Oncorhynchus keta*) but not in coho salmon (*Oncorhynchus kisutch*) (Ryer and Olla 1996). Even in the chum salmon study, however, no differences in agonistic behavior or dominance were found when the fish from localized and scatter-fed regimes were tested in novel laboratory conditions. The enriched rearing environments in the present study, including a combination of submerged structure and localized underwater feed delivery system, resulted in increased competitive ability in two separate novel test environments.

Previous studies (Nielsen 1992; Martel 1996) of cohabiting juvenile coho salmon have found that individuals identified as dominant exhibit higher growth rates than subordinates. In laboratory studies comparing aggressive behavior or dominance relationships between two or more populations (e.g., hatchery versus wild), those populations found to be more aggressive (or dominant) are expected to outcompete less aggressive populations in natural streams (e.g., Swain and Riddell 1990; Berejikian et al. 1996). However, experiments conducted in a laboratory setting may differentially benefit one group or another because of adaptation or acclimation to environmental factors, such as fish density (Fenderson et al. 1968), making inferences about laboratory-derived dominance relationships in growth or survival in natural streams less certain. Huntingford and Garcia de Leaniz (1997) found that the social status of Atlantic salmon in 45-L laboratory aquariums at "moderate" densities (55 fish·m^2) was inversely related to their subsequent growth rates in a laboratory stream channel at lower densities (3.85 fish·m^2). They speculated that within a population, the competitive performance of fish tested under different densities will depend on their individual behavioral profiles. In the present study, the growth performance of fish from the same treatments in a quasi-natural stream (density = 2.8 fish·m^2) supported the results of the dominance experiment in smaller scale flumes (density = 10.6 fish·m^2). Thus, the direction and consistency of results of the dominance and growth experiments suggest that fish reared in enriched tanks would outcompete conventionally reared fish for foraging territories in natural streams.

We did not test whether the enrichment protocols used in the present study produce steelhead that are more similar to wild fish in their behavior. However, the dominance and growth-in-competition advantages of the fish reared in enriched tanks suggest a tendency towards strong territorial behavior, which is consistent with observations of the behavior patterns of wild juvenile steelhead in nature (Edmundson et al. 1968; Everest and Chapman 1972; Berejikian 1995*a*).

Competitive interactions between hatchery-reared and extant wild fish in streams are an important concern in the management of imperiled salmonid populations. The concept of stocking highly competitive fish from enriched hatchery environments, such as those used in this study, could be perceived as potentially detrimental to the more valuable (from a conservation standpoint) wild juveniles. Alternatively, social interactions in the release environment are less likely to be disruptive to wild fish if enriched hatchery environments promote more natural development of social behavior (see Bachman 1984), provided that hatchery-reared fish are also (*i*) derived from locally adapted wild broodstock (Busack and Currens 1995), (*ii*) restocked at densities within the carrying capacity of the target stream (Reisenbichler 1996), and (*iii*) within the size range of wild fish to minimize size-related effects on competition (Abbott et al. 1985; Holtby et al. 1993; Berejikian et al. 1996).

Steelhead hatchery programs designed to produce fish for harvest most commonly release age-1 smolts because survival to that stage in the hatchery is substantially greater than for fish released as age-0 presmolts. Alternatively, conservation hatchery programs designed to maintain the viability and genetic integrity of imperiled wild populations must also place strong emphasis on reducing the potential for developmental effects of culture (Flagg and Nash 1999), which cause departures from the wild state and increase with duration in captivity (Fleming et al. 1994). Therefore, the trade-offs between increased survival in culture and unnatural behavioral (Berejikian et al. 1996), morphological (Fleming et al. 1994), or physiological development (Beckman and Dickhoff 1998) must be considered for each particular program. Although results of the the present study are most relevant to conservation hatchery programs releasing presmolts, the developmental differences in competitive behavior occurred after a short 49- to 78-day rearing period and might be expected to become more substantial after longer rearing periods.

Hatchery programs may also result in genetic divergence of survival-related characteristics of steelhead (Reisenbichler and McIntyre 1977; Berejikian 1995*b*). Differences in resource acquisition and trade-offs between competition and predator avoidance between natural and hatchery environments were theorized as the cause of demonstrated divergence in genetically based behavioral traits of hatchery and wild populations of coho salmon (Swain and Riddell 1990) and steelhead (Berejikian et al. 1996). It may be impossible to completely eliminate the differential selection regimes experienced by cultured and wild salmonids (Waples 1999). However, rearing fish in more naturalistic environments could result in hatchery fish that behave and integrate into

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the postrelease (natural) environment in a manner more similar to wild fish, thereby exposing them to more natural selective pressures.

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References

Abbott, J.C., and Dill, L.M. 1985. Patterns of aggressive attack in juvenile steelhead trout (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci. **42**: 1702–1706.

Abbott, J.C., Dunbrack, R.L., and Orr, C.D. 1985. The interaction of size and experience in dominance relationships of juvenile steelhead trout (*Salmo gairdneri*). Behaviour, **92**: 241–253.

Bachman, R.A. 1984. Foraging behavior of free-ranging wild and hatchery brown trout in a stream. Trans. Am. Fish. Soc. **113**: 1–32.

Beckman, B.R., and Dickhoff, W.W. 1998. Plasticity of smolting in spring chinook salmon: relation to growth and insulin-like growth factor I. J. Fish Biol. **53**: 808–826.

Berejikian, B.A. 1995*a*. The effects of hatchery and wild ancestry and environmental factors on the behavioural development of steelhead trout (*Oncorhynchus mykiss*) fry. Ph.D. dissertation, Univeristy of Washington, Seattle, Wash.

Berejikian, B.A. 1995*b*. The effects of hatchery and wild ancestry and experience on the ability of steelhead trout (*Oncorhynchus mykiss*) fry to avoid a benthic predator. Can. J. Fish. Aquat. Sci. **52**: 2476–2482.

Berejikian, B.A., Mathews, S.B., and Quinn, T.P. 1996. The effects of hatchery and wild ancestry and rearing environments on the development of agonistic behavior in steelhead trout (*Oncorhynchus mykiss*) fry. Can. J. Fish. Aquat. Sci. **53**: 2004–2014.

Berejikian, B.A., Smith, R.J.F., Tezak, E.P., Schroder, S.L., and Knudsen, C.M. 1999. Chemical alarm signals and complex hatchery rearing habitats affect anti-predator behaviour and survival of chinook salmon (*Oncorhynchus tshawytscha*) juveniles. Can. J. Fish. Aquat. Sci. **56**: 830–838.

Bisson, P.A., Sullivan, K., and Nielsen, J.L. 1988. Channel hydraulics, habitat use, and body form of juvenile coho salmon, steelhead, and cutthroat trout in streams. Trans. Am. Fish. Soc. **117**: 262–273.

Bugert, R.M., Bjornn, T.C., and Meehan, W.R. 1991. Summer habitat use by young salmonids and their responses to cover and predators in a small southeast Alaska stream. Trans. Am. Fish. Soc. **120**: 474–485.

Busack, C.A., and Currens, K.P. 1995. Genetic risks and hazards in hatchery operations: fundamental concepts and issues. Am. Fish. Soc. Symp. **15**: 71–80.

Dickson, T.A., and MacCrimmon, H.R. 1982. Influence of hatchery experience on growth and behaviour of juvenile Atlantic salmon (*Salmo salar*) within allopatric and sympatric stream populations. Can. J. Fish. Aquat. Sci. **39**: 1059–1064.

Edmundson, E., Everest, F.E., and Chapman, D.W. 1968. Permanence of station in juvenile chinook salmon and steelhead trout. J. Fish. Res. Board Can. **25**: 1453–1464.

Everest, F.H., and Chapman, D.W. 1972. Habitat selection and spatial interaction by juvenile chinook salmon and steelhead trout in two Idaho streams. J. Fish. Res. Board Can. **29**: 91–100.

Fenderson, O.C., Everhart, W.H., and Muth, K.M. 1968. Comparative agonistic and feeding behaviour of hatchery-reared and wild salmon in aquaria. J. Fish. Res. Board Can. **25**: 1–14.

Flagg, T.A., and Nash, C.E. (*Editors*). 1999. A conceptual framework for conservation hatchery strategies for Pacific salmonids. NOAA Tech. Memo. NMFS-NWFSC-38.

Flagg, T.A., Mahnken, C.V.W., and Johnson, K.A. 1995. Captive broodstocks for recovery of Snake River sockeye salmon. Am. Fish. Soc. Symp. **15**: 81–90.

Fleming, I.A., Jonsson, B., and Gross, M.R. 1994. Phenotypic divergence of sea-ranched, farmed, and wild salmon. Can. J. Fish. Aquat. Sci. **51**: 2808–2824.

Fleming, I.A., Lamberg, A., and Jonsson, B. 1997. Effects of early experience on the reproductive performance of Atlantic salmon. Behav. Ecol. **8**: 470–480.

Grant, J.W.A., and Noakes, D.L.G. 1987. A simple model of optimal territory size for drift-feeding fish. Can. J. Zool. **65**: 270–276.

Hartman, G.F. 1963. Observations of behavior of juvenile brown trout in a stream aquarium during winter and spring. J. Fish. Res. Board Can. **20**: 769–787.

Hartman, G.F. 1965. The role of behaviour in the ecology and interaction of underyearling coho salmon (*Oncorhynchus kisutch*) and steelhead trout (*Salmo gairdneri*). J. Fish. Res. Board Can. **22**: 1035–1018.

Hilborn, R. 1992. Hatcheries and the future of salmon in the Northwest. Fisheries (Bethesda), **17**: 5–8.

Holtby, L.B., Swain, D.P., and Allan, G.M. 1993. Mirror-elicited agonistic behaviour and body morphology as predictors of dominance status in juvenile coho salmon (*Oncorhynchus kisutch*). Can. J. Fish. Aquat. Sci. **50**: 676–684.

Huntingford, F.A., and Garcia de Leaniz, C. 1997. Social dominance, prior residence and the acquisition of profitable feeding sites in juvenile Atlantic salmon. J. Fish Biol. **51**: 1009–1014.

Keeley, E.R., and McPhail, J.D. 1998. Food abundance, intruder pressure, and body size as determinants of territory size in juvenile steelhead trout (*Oncorhynchus mykiss*). Behaviour, **135**: 65–82.

Keenleyside, M.H.A., and Yamamoto, R.T. 1962. Territorial behaviour of juvenile Atlantic salmon (*Salmo salar*). Behaviour, **19**: 139–169.

Li, H.W., and Brocksen, R.W. 1976. Approaches to the analysis of energetic costs of intraspecific competition for space by rainbow trout (*Salmo gairdneri*). J. Fish Biol. **11**: 329–341.

Martel, G. 1996. Growth rate and influence of predation risk on territoriality in juvenile coho salmon (*Oncorhynchus kisutch*). Can. J. Fish. Aquat. Sci. **53**: 660–669.

Maynard, D.J., Flagg, T.A., and Mahnken, C.V.W. 1995. A review of seminatural culture strategies for enhancing the postrelease survival of anadromous salmonids. Am. Fish. Soc. Symp. **15**: 307–314.

Maynard, D.J., Flagg, T.A., Mahnken, C.V.W., and Schroder, S.L. 1996. Natural rearing technologies for increasing postrelease survival of hatchery-reared salmon. Bull. Natl. Res. Inst. Aquacult. Suppl. **2**: 71–77.

Mesick, C.F. 1988. Effects of food and cover on numbers of Apache and brown trout establishing residence in artificial stream channels. Trans. Am. Fish. Soc. **117**: 421–431.

Nielsen, J. 1992. Microhabitat-specific foraging behavior, diet, and growth of juvenile coho salmon. Trans. Am. Fish. Soc. **124**: 617–634.

Reisenbichler, R.R. 1996. Genetic Factors contributing to declines of anadromous salmonids in the Pacific Northwest. *In* Pacific salmon

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and their ecosystems. *Edited by* D. Stouder and R. Naiman. Chapman and Hall, Inc., New York. pp. 223–244.

Reisenbichler, R.R., and McIntyre, J.D. 1977. Genetic differences in growth and survival of juvenile hatchery and wild steelhead trout, *Salmo gairdneri*. J. Fish. Res. Board Can. **34**: 123–128.

Ryer, C.H., and Olla, B.L. 1991. Agonistic behavior in a schooling fish: form, function and ontogeny. Environ. Biol. Fishes, **31**: 355–363.

Ryer, C.H., and Olla, B.L. 1995. The influence of food distribution upon the development of aggressive and competitive behaviour in juvenile chum salmon, *Oncorhynchus keta*. J. Fish Biol. **46**: 264–272.

Ryer, C.H., and Olla, B.L. 1996. Growth depensation and aggression in laboratory reared coho salmon: the effect of food distribution and ration size. J. Fish Biol. **48**: 686–694.

Swain, D.P., and Riddell, B.E. 1990. Genetic variation in agonistic behaviour of juveniles between hatchery and wild stocks of coho salmon (*Oncorhynchus kisutch*). Can. J. Fish. Aquat. Sci. **47**: 566–573.

Symons, P.K. 1968. Increase in aggression and strength of the social hierarchy among juvenile Atlantic salmon deprived of food. J. Fish. Res. Board Can. **25**: 2387–2401.

Waples, R.S. 1999. Dispelling some myths about hatcheries. Fisheries (Bethesda), **12**: 12–21.

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Population genetics of the zebra mussel, *Dreissena polymorpha* (Pallas): local allozyme differentiation within midwestern lakes and streams

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Abstract: Several aquatic invertebrates with free-swimming larvae have paradoxically demonstrated fine-scale genetic heterogeneity. In this study, we tested for genetic heterogeneity in an exotic freshwater bivalve, the zebra mussel, *Dreissena polymorpha* (Pallas), which like many marine molluscs has a free-swimming larval stage. Zebra mussels were collected from 22 sites in the Great Lakes and from a small inland lake complex in southwestern Michigan and scored for 13 allozyme loci. Sites were sampled in a hierarchical fashion to assess the spatial scale of genetic variation. Zebra mussel populations exhibited significant genetic heterogeneity on a local scale within lakes, even though populations remained homogenous on a larger regional scale between lakes or lake complexes. The allozyme loci that exhibited heterogeneity differed from lake to lake. Populations also displayed significant heterozygote deficiencies from Hardy–Weinberg expectations for a majority of loci, implying population subdivision and (or) inbreeding on a fine scale. Our results suggest that local genetic differentiation for zebra mussels is both spatially and temporally fluid and is the product of stochastic processes, such as spawning asynchrony and uneven mixing of larval cohorts, rather than natural selection.

Résumé : On a paradoxalement observé une hétérogénéité génétique fine chez plusieurs invertébrés aquatiques à larves nageuses. Dans la présente étude, nous avons examiné s'il y a hétérogénéité génétique chez le bivalve exotique d'eau douce, la moule zébrée, *Dreissena polymorpha* (Pallas), qui, comme de nombreux mollusques marins, passe par un stade de larve nageuse. On a recueilli des moules zébrées à 22 sites des Grands Lacs et dans un petit complexe lacustre enclavé du sud-ouest du Michigan pour en examiner 13 loci d'allozymes. On a échantillonné les sites hiérarchiquement pour évaluer l'échelle spatiale de la variation génétique. Les populations de moules zébrées ont montré une hétérogénéité génétique significative à l'échelle locale à l'intérieur des lacs, mais demeuraient homogènes à l'échelle régionale entre lacs ou complexes lacustres. Les loci d'allozymes qui étaient hétérogènes n'étaient pas les mêmes d'un lac à l'autre. On a aussi observé chez les populations des réductions cas importantes de l'hétérozygotie par rapport aux prévisions de la loi de Hardy–Weinberg pour une majorité de loci, ce qui laisse croire en une subdivision des populations et (ou) à une endogamie à petite échelle. Nos résultats montrent que la différenciation génétique locale des moules zébrées est spatialement et temporellement fluide, et qu'elle est le produit de processus stochastiques, associés à l'asynchronie des pontes ou au mélange inégal des cohortes de larves, plutôt que celui de la sélection naturelle.

[Traduit par la Rédaction]

Introduction

An aquatic species with large population numbers and a free-swimming larval stage should be genetically homogenous across great distances within connected bodies of water (Palumbi 1995). Genetic shifts that occur in local populations, whether due to genetic drift or natural selection, should be quickly erased by gene flow from surrounding populations. Yet this does not always appear to be the case, especially in marine molluscs (Johnson and Black 1984; Watts et al. 1990; Hedgecock 1994; Edmands et al. 1996; David et al. 1997; reviewed in Palumbi 1995 and Mitton 1997).

Sources of genetic heterogeneity fall into two general categories, those primarily due to deterministic processes (i.e., natural selection) and those due to stochastic processes (i.e., genetic drift). In the deterministic category, genetic variation (usually for a single locus) is related to a particular environmental variable. An example of this type of population structure is the cline for leucine aminopeptidase (LAP) among populations of the blue mussel, *Mytilus edulis* (Koehn et al. 1980). Hilbish and Koehn (1985*a*, 1985*b*) have demonstrated that selection against certain LAP genotypes is sufficient to maintain allele frequency differences between estuarine and marine populations of *M. edulis*, despite the exchange of larvae between the two habitats. In the stochastic category, population structure is characterized by an unstable, chaotic pattern of genetic variation across populations (Johnson and Black 1984; Watts et al. 1990; Johnson et al.

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1993; Kordos and Burton 1993). This pattern is suggestive of genetic drift caused by spatial and temporal variation in juvenile recruitment (Watts et al. 1990; Johnson et al. 1993; Kordos and Burton 1993).

The zebra mussel, *Dreissena polymorpha* (Pallas), was introduced into North American freshwater ecosystems (originally the Great Lakes) during the mid-1980s via ballast discharge from European vessels (Hebert et al. 1989). Zebra mussels have a free-swimming planktonic larval stage that develops into a sessile, benthic adult, an unusual trait for freshwater molluscs but common in marine molluscs. This exotic mussel therefore provides a model to test whether the same population genetics that characterize many marine invertebrates also exist for a freshwater benthic organism.

Many previous studies have focused on the ecological and economic impacts of zebra mussels on North American aquatic habitats, but less is known about the population genetics of this species. *Dreissena polymorpha* populations in the United States and Canada have been found to contain extensive allozyme polymorphism, characteristic of species with large effective population sizes (Hebert et al. 1989; Garton and Haag 1991; Boileau and Hebert 1993; Haag and Garton 1995; Marsden et al. 1995). Nevertheless, significant geographic variation in allele frequency has been reported among populations within Lakes St. Clair, Erie, Michigan, and Ontario (Boileau and Hebert 1993; Marsden et al. 1995). The cause for this variation (i.e., deterministic or stochastic) is unclear. Russian researchers have claimed that allele frequencies for three allozyme loci (esterase-D, LAP, and malic enzyme) in zebra mussel populations surrounding the Chernobyl power plants were correlated with water temperature (Fetisov et al. 1992). It is therefore possible that water temperature (or an environmental factor correlated with water temperature) could explain the genetic heterogeneity present among North American *D. polymorpha* populations.

Here, we report on an extensive genetic survey of *D. polymorpha* populations collected from the Laurentian Great Lakes and from a small inland lake complex in southwestern Michigan (Eagle Lake complex). Our objective was to characterize the spatial scale (e.g., between complexes of lakes, among lakes, within lakes) over which genetic differentiation occurs for zebra mussels. We also tested for the possible effects of water temperature on allozyme variation in Milwaukee Harbor, Lake Michigan, Wis., to determine whether any differentiation observed could be due to natural selection.

Materials and methods

Collection sites and methods

Zebra mussels were collected from two lake complexes: the Laurentian Great Lakes and the Eagle Lake complex (an inland lake complex located in the St. Joseph River basin in southwestern Michigan; see Fig. 1 and accompanying legend for information on collection sites and sample sizes). For the Laurentian Great Lakes, we collected zebra mussels from three sites each in Lakes Erie, Michigan, and Ontario during the summer of 1995 (Fig. 1A). Within the Eagle Lake complex, we collected zebra mussels from three sites each in Eagle Lake (surface area = 153 ha), Christiana Lake (surface area = 72 ha), and Christiana Creek during the summer of 1994 (Fig. 1C). A "site" refers to a 10-m^2 collecting area within a lake. To measure the amount of variation present on a microgeographic scale, one 10-m^2 site within Eagle Lake (EL1) (Fig. 1C) was divided into three subsites to examine microgeographic differentiation.

Our sampling design allowed for a four-level hierarchical analysis of population structure: (*i*) between complexes of lakes (i.e., the Great Lakes versus the Eagle Lake complex), (*ii*) among lakes within a complex (e.g., Lake Erie versus Lake Michigan versus Lake Ontario), (*iii*) among sites within lakes (e.g., site CL1 versus site CL2 versus site CL3 within Christiana Lake), and (*iv*) among microgeographic subpopulations within one site (i.e., EL1).

To test for the effects of temperature on North American zebra mussel populations, we collected adult zebra mussels from four sites in Lake Michigan near Milwaukee (Fig. 1B). Two sites (MH1 and MH2) were located in the industrial inner harbor of the city, while the remaining two sites (MH3 and MH4) were located in the outer harbor, separated from Lake Michigan by a breakwall. The outer harbor receives a greater influx of lake water, while the inner harbor is warmed by industrial outputs. As a result, temperatures differ between the two sets of sites (A.S. Brooks, University of Wisconsin-Milwaukee, personal communication). Water temperatures on the day of collection (23 May 1996) were 17.0 and 16.3°C for sites MH1 and MH2, respectively, and 11.0 and 8.9°C for sites MH3 and MH4, respectively.

Zebra mussels from the Great Lakes and the Eagle Lake complex were collected from hard substrates at depths of 0.5–1.5 m. Collections were made at random within a 10 × 10 m area. Individuals were transported to the laboratory on ice (nearby sites) or were immediately frozen on dry ice (distant sites). Samples were stored at –80°C prior to electrophoresis. Zebra mussels from Milwaukee Harbor were scraped from harbor walls at all four sites and immediately frozen on dry ice.

Genetic techniques

Soft tissue was removed from zebra mussel shells and a portion was homogenized in 15 µL of grinding buffer for allozyme analysis. Zebra mussels from the Eagle Lake complex and the Great Lakes were scored for a total of nine enzyme systems using standard starch gel electrophoresis (Murphy et al. 1990). We resolved a total of 13 different loci for these systems (Table 1). Individuals from the four Milwaukee Harbor sites were scored for a subset of these loci: ACO, IDHP, MDH1, ODH, and GPI. Five of the allozymes, ACO, IDHP, MDH1, ODH, and GPI, were resolved using a Tris–citrate electrode buffer system (pH = 6.3). The remaining loci, EST1, EST2, MEP, MDH2, and the peptidases, were resolved using a Tris–citrate electrode buffer system (pH = 8.0). Stain recipes were those of Murphy et al. (1990).

Data analysis

Populations were tested for allele frequency variation using *G* heterogeneity tests. Rare alleles were pooled as necessary to ensure that average expected frequency within cells was >6 (Zar 1999). Tablewide significance was determined using a sequential Bonferroni procedure (Holm 1979; Rice 1989). The *F* statistics (F_{IT}, F_{IS}, and F_{ST}) and expected and observed heterozygosities (H_e and H_o) were calculated using the Genetic Data Analysis system of Lewis and Zaykin (1996) based on the methods of Weir and Cockerham (1984). The *F* statistics were derived using a hierarchical design (Weir 1996) in which the total reduction in heterozygosity F_{IT} was partitioned into three components: (*i*) the portion due to local inbreeding within sites, F_{IS}, (*ii*) the portion due to differentiation among sampling sites within lakes, $F_{(ST)S}$, and (*iii*) the portion due to between-lake differentiation, $F_{(ST)I}$. We confined this analysis to the 18 study sites for which zebra mussels were scored for all 13 allozymes. Ninety-five percent confidence intervals were calculated for the *F* statistics by bootstrapping across loci (number of replications = 10 000; Weir 1996). Values for aver-

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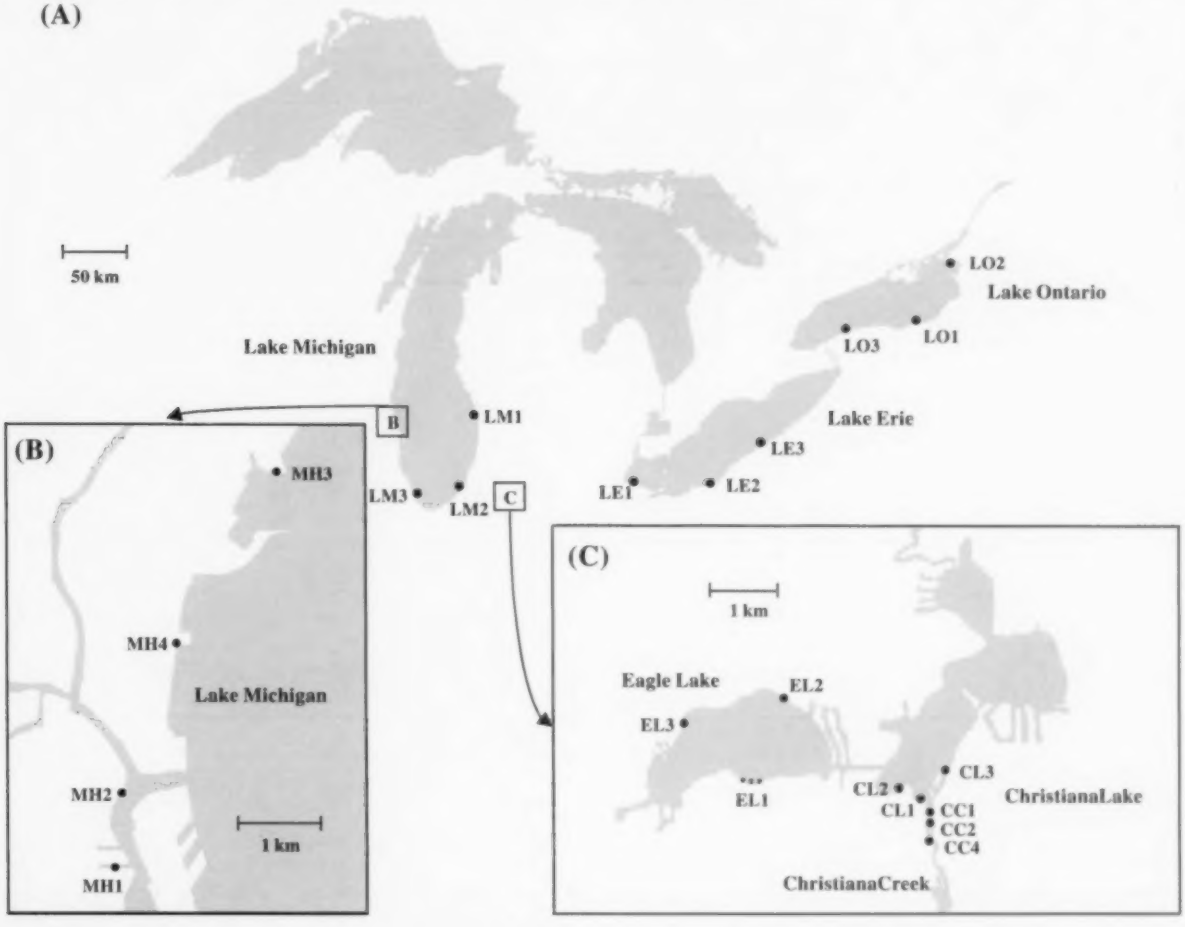
Fig. 1. (A) Collection sites and sample sizes (n, the number of mussels genetically scored) in the Great Lakes. Lake Michigan samples were from Douglas, Mich. (LM1, n = 50), New Buffalo, Mich. (LM2, n = 49), and Chicago, Ill. (LM3, n = 50). Lake Erie samples were from Maumee, Ohio (LE1, n = 77), Huron, Ohio (LE2, n = 50), and Headlands Beach, Ohio (LE3, n = 48). Lake Ontario samples were from Sodus Point, N.Y. (LO1, n = 50), Cape Vincent, N.Y. (LO2, n = 50), and 30 Mile Point, N.Y. (LO3, n = 49). (B) Collection sites in Milwaukee Harbor, Lake Michigan, Wis. Sites MH1 (n = 50) and MH2 (n = 50) were located within the warm industrial inner harbor of the city, and MH3 (n = 50) and MH4 (n = 50) were located in the colder, outer harbor. (C) Collection sites within the Eagle Lake Complex. Zebra mussels were collected from three sites each in Eagle Lake (EL1, n = 432; EL2, n = 142; EL3, n = 142), Christiana Lake (CL1, n = 127; CL2, n = 128; CL3, n = 124), and Christiana Creek (CC1, n = 128; CC2, n = 142; CC4, n = 138). One site in Eagle Lake (EL1) was subdivided into three microgeographic sites.



age heterozygosity (H) and average number of alleles per locus (A) are reported as mean ± SE.

Results

High levels of allozyme polymorphism existed within all zebra mussel populations. The average heterozygosity (H) for the 13 loci resolved in the study was 0.36 ± 0.003 in the Eagle Lake complex and 0.38 ± 0.005 in the Great Lakes (Table 2). The average number of alleles per locus (A) was 3.3 ± 0.04 in the Eagle Lake complex and 3.1 ± 0.06 in the Great Lakes (Table 2). One locus, GPI, was extremely polymorphic, possessing 10 different electrophoretic alleles across all populations (Table 1). Over 69% of all individuals scored for this locus in the Eagle Lake complex were heterozygotes, while 76% of Great Lakes individuals were heterozygotes (Table 2). Nonetheless, there was a general deficiency of heterozygotes from Hardy–Weinberg genotypic expectations at all sites (Table 2), suggesting population subdivision and local inbreeding within lakes. Values of F_{IS} were significantly > 0 for seven loci (ACO, EST2, ODH, PEPD, PEPGEN1, PEPGEN3, and GPI) (Table 2). Only MDH2 displayed a significant excess of heterozygotes (Table 2).

Allele frequencies were similar across lakes (K.M. Lewis et al., unpublished data, available upon request). Values of $F_{(ST)P}$ were not significantly > 0 between the Great Lakes and the Eagle Lake complex, among the Great Lakes, or among lakes and the stream of the Eagle Lake complex (Table 3). There was a slight but significant reduction in hetero-

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Table 1. List of enzyme systems resolved giving the abbreviations, enzyme commission numbers (EC code), metabolic function, and number of alleles resolved for each enzyme.

Enzyme locus	Abbreviation	EC code	Metabolic function	No. alleles
Aconitase	ACO	4.2.1.3	Citric acid cycle	3
Esterase (two isozymes)	EST1	3.1.1.1	Carboxylic ester	3
	EST2		Hydrolysis	2
Isocitrate dehydrogenase	IDHP	1.1.1.42	Citric acid cycle	5
Malate dehydrogenase (two isozymes)	MDH1	1.1.1.37	Citric acid cycle	3
	MDH2			3
Malic enzyme	MEP	1.1.1.40	Fatty acid oxidation	3
Octopine dehydrogenase	ODH	1.5.1.11	Anaerobic respiration	3
Peptidase-D (Phe–Pro)	PEPD	3.4.13.9	Peptide hydrolysis	4
Peptidase general (Leu–Ala) (three isozymes)	PEPGEN1	3.4.11	Peptide hydrolysis	4
	PEPGEN2			3
	PEPGEN3			4
Phosphoglucose isomerase	GPI	5.3.1.9	Glycolysis	10

Table 2. Average heterozygosity ($H \pm$ SE) and average number of alleles ($A \pm$ SE) per locus for the Great Lakes and Eagle Lake complex.

	Great Lakes		Eagle Lake complex		
Locus	H	A	H	A	F_{IS}
ACO	0.34±0.011	3.0±0.00	0.36±0.011	3.0±0.00	0.055±0.023*
EST1	0.28±0.024	2.1±0.17	0.24±0.008	2.3±0.11	0.005±0.025
EST2	0.43±0.031	2.0±0.00	0.38±0.011	2.0±0.00	0.144±0.024*
IDHP	0.37±0.026	2.7±0.29	0.44±0.016	3.0±0.24	0.032±0.017
MDH1	0.46±0.023	2.3±0.17	0.48±0.020	2.7±0.17	0.001±0.031
MDH2	0.30±0.020	3.0±0.00	0.24±0.011	3.0±0.00	–0.046±0.020*
MEP	0.02±0.005	1.8±0.18	0.04±0.007	2.6±0.15	0.022±0.040
ODH	0.21±0.018	3.0±0.00	0.21±0.012	3.0±0.00	0.212±0.031*
PEPD	0.61±0.019	3.6±0.15	0.52±0.022	4.3±0.18	0.051±0.025*
PEPGEN1	0.34±0.024	2.9±0.24	0.27±0.011	2.8±0.20	0.070±0.026*
PEPGEN2	0.42±0.019	3.0±0.00	0.39±0.006	3.0±0.00	0.028±0.023
PEPGEN3	0.37±0.023	3.9±0.11	0.41±0.018	4.1±0.11	0.198±0.030*
GPI	0.76±0.023	6.9±0.40	0.69±0.016	7.2±0.26	0.049±0.014*
Overall	0.38±0.005	3.1±0.06	0.36±0.003	3.3±0.04	0.067±0.019*

Note: Also given are F_{IS} values (±1 SD) estimated for loci across sites by the method of Weir and Cockerham (1984). Standard deviations for F_{IS} were determined by jackknifing over populations. Negative values reflect an excess of heterozygotes and positive values a deficiency compared with Hardy–Weinberg expectations. *$P < 0.05$ that $F_{IS} \neq 0$, as determined by jackknifing over populations.

zygosity in the Eagle Lake complex relative to the Great Lakes (Wilcoxon signed rank test, $Z = 1.994$, $P = 0.045$) (Table 2).

Despite the lack of broad-scale geographic variation between lake complexes and among lakes, significant genetic differentiation existed on both local and microgeographic scales within lakes. Thirteen out of 89 single-locus G tests displayed significant allele frequency heterogeneity among sites within lakes (Table 4). Three of these tests were significant on a tablewide basis (PEPD (Eagle Lake), PEPGEN3 (Christiana Creek), and GPI (Christiana Lake)). The 13 significant tests were distributed across seven different loci, and at least one locus was significant in each lake surveyed, except for Lake Michigan (Table 4). The F statistics indicated significant variation among all sites considered together ($F_{(ST)S} = 0.0024$), sites within each Great Lake ($F_{(ST)S} = 0.0063$), and sites within the Eagle Lake complex ($F_{(ST)S} = 0.0027$) (Table 3). Genetic differentiation was as great among sites within Eagle Lake, Christiana Lake, or Christiana Creek ($F_{(ST)S} = 0.0027$) as across the entire Great Lakes ($F_{(ST)S} = 0.0024$). Finally, four loci displayed significant allele frequency differences among the three microgeographic subpopulations collected within a 10-m^2 area in Eagle Lake (Table 4).

Local genetic variation was not correlated with water temperature among the Milwaukee Harbor sites (K.M. Lewis et al., unpublished data). The warm, inner Milwaukee harbor populations did not differ significantly in allele frequencies from the colder, outer harbor populations for any locus. No significant spatial heterogeneity was found among these four sites.

Discussion

The pattern of allozyme differentiation for the freshwater mussel *D. polymorpha* is similar to that observed for many marine invertebrates (e.g., Johnson and Black 1984; Watts et al. 1990; Johnson et al. 1993; Kordos and Burton 1993;

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Table 3. *F* statistics for the Great Lakes and Eagle Lake complex calculated according to the method of Weir and Cockerham (1984).

Sites compared	F_{IT}	F_{IS}	$F_{(ST)P}$	$F_{(ST)S}$
All sites[a]	0.0719*	0.0674*	0.0014	0.0024*
Great Lakes[b]	0.0472*	0.0412	0.0025	0.0063*
Eagle Lake complex[c]	0.0779*	0.0754*	0.0006	0.0027*

Note: $*P < 0.05$ as determined by bootstrap analysis of 10 000 replicates.

[a]Analysis for the Great Lakes and Eagle Lake complex combined; $F_{(ST)P}$ quantifies the level of genetic differentiation between the Great Lakes and Eagle Lake complex, while $F_{(ST)S}$ is a measure of differentiation within each complex of lakes.

[b]Analysis for the Great Lakes sites; $F_{(ST)P}$ quantifies the level of genetic differentiation among the Great Lakes, while $F_{(ST)S}$ is a measure of differentiation among sites within lakes.

[c]Analysis of the Eagle Lake complex; $F_{(ST)P}$ quantifies the level of genetic differentiation among Eagle Lake, Christiana Lake, and Christiana Creek, while $F_{(ST)S}$ is a measure of differentiation among sites within each lake and the creek.

Table 4. Loci displaying significant allele frequency variation among sites within lakes as determined with *G* heterogeneity tests.

Locus	Location	*G*	df	*P*
IDHP	Lake Erie	6.78	2	0.0348
IDHP	Christiana Lake	6.70	2	0.0367
MDH1	Lake Ontario	6.64	2	0.0347
MDH2	Lake Erie	8.40	2	0.0105
MDH2	Christiana Creek	8.34	2	0.0147
MDH2	Eagle Lake	8.81	4	0.0341
ODH	Lake Erie	9.64	2	0.0134
ODH	Eagle Lake subsites	14.00	4	0.0070
PEPD	Eagle Lake	22.26	4	0.0001*
PEPD	Eagle Lake subsites	10.40	4	0.0287
PEPGEN1	Eagle Lake subsites	5.81	2	0.0498
PEPGEN3	Lake Erie	12.26	4	0.0230
PEPGEN3	Christiana Creek	33.95	6	0.0001*
PEPGEN3	Eagle Lake	19.69	6	0.0026
PEPGEN3	Eagle Lake subsites	13.03	6	0.0492
GPI	Christiana Creek	16.13	6	0.0146
GPI	Christiana Lake	30.51	6	0.0002*

Note: $*P < 0.05$ on a tablewide basis as determined with the sequential Bonferroni procedure.

Hedgecock 1994; Edmands et al. 1996). We found limited allele frequency variation for zebra mussels at a broad geographic scale among regions or lakes, consistent with organisms having a great dispersal capacity. However, significant genetic differentiation was observed locally within lakes. Furthermore, these differences persisted even on a microgeographic scale within a 10-m^2 area at one site. Allele frequency differences within lakes were not large in magnitude. Significant frequency differences for the common allele at a locus averaged 0.104 (range 0.043–0.160) among sites within lakes and 0.0639 (range 0.0419–0.0906) among the microgeographic sites at EL1 (K.M. Lewis et al., unpublished data). Consequently, the corresponding F_{ST} values were not particularly high (e.g., 0.0027 for the Eagle Lake complex and 0.0063 within the Great Lakes). Nevertheless, this genetic variation is significant, which is surprising given the presumed high potential for population mixing of *D. polymorpha* as veligers.

The small but significant reduction in heterozygosity observed between zebra mussel populations of the Great Lakes and the Eagle Lake complex may indicate that a population bottleneck occurred during the original colonization of the Eagle Lake complex. Zebra mussels were presumably introduced into the Eagle Lake complex from the Great Lakes by recreational boaters ca. 1991, perhaps as a single event (Horvath et al. 1996). However, there was little genetic differentiation between the two sets of lakes, and virtually all alleles were present in both sets of lakes. Consequently, there is no strong evidence for a founder effect during the colonization of the Eagle Lake complex.

One possible explanation for the pattern of genetic differentiation among *D. polymorpha* populations within lakes is natural selection. Strong selection tied to differences in environmental conditions among sampling sites could counteract gene flow, even if settling larvae were genetically homogenous. Our findings, however, do not support the selection hypothesis. First, allozyme frequencies did not differ between populations at different water temperatures in our study. Contrary to reports from Chernobyl, Russia (Fetisov et al. 1992), we found no evidence for thermal selection on zebra mussels in Milwaukee Harbor. Second, although significant local heterogeneity was found for a number of loci, the set of loci showing variation differed among lakes. It seems unlikely that multiple allozyme loci would be under such diverse selection pressures in adjacent and interconnected bodies of water such as the Eagle Lake complex.

A more plausible explanation for the pattern of genetic differentiation, which we term the "cohort" hypothesis, centers on the population dynamics of zebra mussels. Although zebra mussels are broadcast spawners (Ludyanskiy et al. 1993), spawning is not always well synchronized among localities or individuals at sites (Garton and Haag 1993). Effective population sizes are therefore probably orders of magnitude lower than suggested by the often staggering densities of *D. polymorpha*, which can be upwards of 50 000/m^2 (Dermott et al. 1993). As a consequence, allele frequencies can differ among larval cohorts due to genetic drift, with the scale of this effect being inversely proportional to the number of parents contributing to cohorts (Tracey et al. 1975). If conditions are such that larval cohorts do not mix prior to settlement, then local genetic differentiation can result. For instance, effective population sizes in the range of about 160–370 would generate the F_{ST} values observed within lakes in our study (0.0063–0.0027). In addition, if larval cohorts do not tend to mix, then many new recruits at sites will be genetically related. This will heighten levels of inbreeding when these individuals reach sexual maturity and interbreed, further contributing to genetic differences among cohorts. Of course, patterns of mating are likely to be variable through time. Such demographic instability will produce highly dynamic population genetics, as evident in the "chaotic" nature of allozyme loci displaying significant variation within lakes in the current study.

If the dynamics of zebra mussel mating and dispersal are affecting population structure, then there must be both spatial and temporal genetic variation among settling larval cohorts. Unfortunately, technical limitations make it difficult to score zebra mussel larvae for allozymes. Nevertheless, Haag and Garton (1995) were able to score larval populations for

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one locus (GPI) using cellulose acetate electrophoresis. They found that planktonic larvae had different GPI genotype frequencies than parental populations, a difference that they attributed to natural selection. However, given our results, it seems more likely that such differences could be due to population demography rather than to selection. Regardless, resolution of the cohort issue requires the simultaneous scoring of a number of different loci from the same set of individuals. The small size of zebra mussel veligers will always limit the number of allozyme loci that can be resolved. A possible future approach would therefore be to develop molecular DNA markers for *D. polymorpha* based on polymerase chain reaction technology. Such a strategy has recently been used to examine mtDNA variation among samples of larvae from the Pacific oyster, *Crassostrea gigas*, in Dabob Bay, Wash. (Li and Hedgecock 1998). Significant mtDNA haplotype frequency variation was observed among oyster cohorts during a single spawning season. This differentiation was ascribed to genetic drift owing to a large variance in adult reproductive success through the season, similar to what we propose for zebra mussels.

The general deficiency of heterozygotes from Hardy–Weinberg equilibrium could be interpreted as corroborative evidence for population substructuring and inbreeding in zebra mussels, supporting the cohort hypothesis. However, heterozygote deficiencies were not uniform across loci (Q test for heterogeneity of F_{IS} values by metaanalysis = 144.0, $P < 0.0001$, 12 df). It is therefore not possible for a single inbreeding coefficient to explain the observed genotypic deviations by a Wahlund effect. In addition, the rank order of F_{IS} values for loci was consistent across sites (Kendall congruence coefficient (W) = 0.289, $\chi^2 = 62.47$, $P < 0.0001$, 12 df). ODH, PEPGEN1, PEPGEN3, and EST2 generally showed the largest heterozygote deficiencies at sites, while MEP, MDH2, EST1, and PEPD displayed the smallest deviations. These results suggest that other factors, in addition to population subdivision and inbreeding, are contributing to the observed heterozygote deficiencies.

It has recently been suggested that departures of marine invertebrates from Hardy–Weinberg equilibrium could be explained by the presence of allozyme null alleles (Gaffney 1994; Hoare and Beaumont 1995; David et al. 1997). We cannot rule out this possibility for zebra mussels. In fact, we detected a null allele at MDH2 that ranged in frequency from about 0.011 to 0.053 at sites (K.M. Lewis et al., unpublished data). Since this particular null allele was able to dimerize, null heterozygotes gave a distinct double, instead of triple, banding zymogram pattern in gels. Interestingly, MDH2 was the only locus that displayed a significant excess of heterozygosity in our study, perhaps the result of us misscoring MDH2 null homozygotes as unresolved individuals. If the null heterozygotes are removed from consideration, then the F_{IS} value for MDH2 is not significantly different from 0 (–0.022 ± 0.276). The relatively large departures from Hardy–Weinberg proportions observed for the loci ODH, PEPGEN1, PEPGEN3, and EST2 could therefore be due to undetected null alleles. We estimate that null alleles segregating at frequencies of 0.036–0.118 would account for the ODH, PEPGEN1, PEPGEN3, and EST2 results. These estimates are based on the formula $r = D/(2 - D)$, where r is the frequency of the null allele at a locus and D is the heterozygote deficiency (Chakraborty et al. 1993). Of course, this estimate assumes that the deficiencies are only caused by null alleles, which is clearly not the case, as population subdivision also exists. Furthermore, ODH, PEPGEN1, PEPGEN3, and EST2, the loci with the largest heterozygote deficiencies, are all monomers in zebra mussels. Consequently, null alleles would be difficult to detect at these loci, as null heterozygotes would not display an unusual banding pattern but would appear as homozygotes for the alternate allele at the locus. Further work is needed to assess the prevalence of null allozyme alleles in zebra mussel populations.

In conclusion, zebra mussels display significant genetic heterogeneity on a local scale within lakes, even though populations remain relatively homogenous on a larger regional scale between lakes. This pattern of differentiation is similar to that reported for a number of marine invertebrates. We suggest that fine-grained genetic differentiation for zebra mussels is the product of a dynamic population demography rather than natural selection. Additional genetic studies of larvae at the molecular level are needed, however, to verify that local patterns of allozyme variation for zebra mussels are due to asynchrony in spawning and incomplete mixing of veliger cohorts.

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References

Boileau, M.G., and Hebert, P.D.N. 1993. Genetics of the zebra mussel (*Dreissena polymorpha*) in populations from the Great Lakes region and Europe. *In* Zebra mussels: biology, impacts, and control. *Edited by* T.F. Nalepa and D.W. Schloesser. Lewis Publishers, Boca Raton, Fla. pp. 225–238.

Chakraborty, R., De Andrade, M., Daiger, S.P., and Budowle, B. 1993. Apparent heterozygote deficiencies observed in DNA typing data and their implications in forensic applications. Ann. Hum. Genet. **56**: 45–57.

David, P., Perdieu, M.A., Pernot, A.F., and Jarne, P. 1997. Fine-grained spatial and temporal population genetic structure in the marine bivalve *Spisula ovalis*. Evolution, **51**: 1318–1322.

Dermott, R., Mitchell, J., Murray, I., and Fear, E. 1993. Biomass and production of zebra mussels (*Dreissena polymorpha*) in shallow waters of northeastern Lake Erie. *In* Zebra mussels: biology, impacts, and control. *Edited by* T.F. Nalepa and D.W. Schloesser. Lewis Publishers, Boca Raton, Fla. pp. 146–153.

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Edmands, S., Moberg, P.E., and Burton, R.S. 1996. Allozyme and mitochondrial DNA evidence of population subdivision in the purple sea urchin, *Strongylocentrotus purpuratus*. Mar. Biol. **126**: 443–450.

Fetisov, A.N., Rubanovich, A.V., Slipchenko, T.S., and Shevchenko, V.A. 1992. The structure of *Dreissena polymorpha* populations from basins adjacent to the Chernobyl atomic power station. Sci. Total Environ. **112**: 115–124.

Gaffney, P.M. 1994. Heterosis and heterozygote deficiencies in marine bivalves: more light? *In* Genetics and evolution of aquatic organisms. *Edited by* A.R. Beaumont. Chapman and Hall, London, U.K. pp. 146–153.

Garton, D.W., and Haag, W.R. 1991. Heterozygosity, shell length and metabolism in the European mussel, *Dreissena polymorpha*, from a recently established population in Lake Erie. Comp. Biochem. Physiol. **99**: 45–48.

Garton, D.W., and Haag, W.R. 1993. Seasonal reproductive cycles and settlement patterns in western Lake Erie. *In* Zebra mussels: biology, impacts, and control. *Edited by* T.F. Nalepa and D.W. Schloesser. Lewis Publishers, Boca Raton, Fla. pp. 111–128.

Haag, W.R., and Garton, D.W. 1995. Variation in genotype frequencies during the life history of the bivalve, *Dreissena polymorpha*. Evolution, **49**: 1284–1288.

Hebert, P.D.N., Muncaster, B.W., and Mackie, G.L. 1989. Ecological and genetic studies on *Dreissena polymorpha* (Pallas): a new mollusk in the Great Lakes. Can. J. Fish. Aquat. Sci. **46**: 1587–1591.

Hedgecock, D. 1994. Temporal and spatial genetic structure of marine animal populations in the California Current. Calif. Coop. Oceanic Fish. Invest. Rep. **35**: 73–81.

Hilbish, T.J., and Koehn, R.K. 1985*a*. Exclusion of the role of secondary contact in an allele frequency cline in the mussel *Mytilus edulis*. Evolution, **39**: 432–443.

Hilbish, T.J., and Koehn, R.K. 1985*b*. The physiological basis of natural selection at the LAP locus. Evolution, **39**: 1302–1317.

Hoare, K., and Beaumont, A.R. 1995. Effects of an ODH null allele and a GPI low-activity allozyme on shell length in laboratory reared *Mytilus edulis*. Mar. Biol. **123**: 775–780.

Holm, S. 1979. A simple sequentially rejective multiple test procedure. Scand. J. Stat. **6**: 65–70.

Horvath, T.G., Lamberti, G.A., Lodge, D.M., and Perry, W.L. 1996. Zebra mussel dispersal in lake–stream systems: source-sink dynamics? J. North Am. Benthol. Soc. **15**: 564–575.

Johnson, M.S.,and Black, R. 1984. Pattern beneath the chaos: the effect of recruitment on genetic patchiness in an intertidal limpet. Evolution, **38**: 1371–1383.

Johnson, M.S., Holborn, K., and Black, R. 1993. Fine scale patchiness and genetic heterogeneity of recruits of the corallivorous gastropod *Drupella cornus*. Mar. Biol. **117**: 91–96.

Koehn, R.K., Newell, R.I.E., and Immerman, F. 1980. Maintenance of an aminopeptidase allele frequency cline by natural selection. Proc. Natl. Acad. Sci. U.S.A. **77**: 5385–5389.

Kordos, L.M., and Burton, R.S. 1993. Genetic differentiation of Texas Gulf Coast populations of the blue crab *Callinectes sapidus*. Mar. Biol. **117**: 227–233.

Lewis, P.O., and Zaykin, D. 1996. Genetic data analysis: software for the analysis of discrete genetic data. Computer program distributed by the authors. [http://alleyn.eeb.uconn.edu/gda/]

Li, G., and Hedgecock, D. 1998. Genetic heterogeneity, detected by PCR-SSCP, among samples of larval Pacific oysters (*Crassostrea gigas*) supports the hypothesis of large variance in reproductive success. Can. J. Fish. Aquat. Sci. **55**: 1025–1033.

Ludyanskiy, M.L., McDonald, D., and MacNeil, D. 1993. Impact of the zebra mussel, a bivalve invader. BioScience, **43**: 533–544.

Marsden, J.E., Spidle, A., and May, B. 1995. Genetic similarities among zebra mussel populations within North America and Europe. Can. J. Fish. Aquat. Sci. **52**: 836–847.

Mitton, J.B. 1997. Selection in natural populations. Oxford University Press, New York.

Murphy, R.W., Sites, J.W., Jr., Buth, D.G., and Haufler, C.H. 1990. Proteins I: isozyme electrophoresis. *In* Molecular systematics. *Edited by* D.M. Hillis and C. Moritz. Sinauer Associates, Inc., Sunderland, Mass. pp. 45–126.

Palumbi, S. 1995. Using genetics as an indirect estimator of larval dispersal. *In* Ecology of marine invertebrate larvae. *Edited by* L. McEdward. CRC Press, Boca Raton, Fla. pp. 369–387.

Rice, W.R. 1989. Analyzing tables of statistical tests. Evolution, **43**: 223–225.

Tracey, M.L., Bellet, N.F, and Gravem, C.D. 1975. Excess allozyme homozygosity and breeding population structure in the mussel *Mytilus californianus*. Mar. Biol. **32**: 303–311.

Watts, R.J., Johnson, M.S., and Black, R. 1990. Effects of recruitment on genetic patchiness in the urchin *Echinometra mathaei* in western Australia. Mar. Biol. **105**: 145–151.

Weir, B.S. 1996. Genetic data analysis II: methods for discrete population genetic data. Sinauer Associates, Inc., Sunderland, Mass.

Weir, B.S., and Cockerham, C.C. 1984. Estimating *F*-statistics for the analysis of population structure. Evolution, **38**: 1358–1370.

Zar, J.H. 1999. Biostatistical analysis. Prentice Hall, Inc., Upper Saddle River, N.J.

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PERSPECTIVE

Distribution shifts and overfishing the northern cod (*Gadus morhua*): a view from the ocean

G.A. Rose, B. deYoung, D.W. Kulka, S.V. Goddard, and G.L. Fletcher

Abstract: Research on northern cod (*Gadus morhua*) from 1983 to 1994 indicated that a southward shift in distribution in the early 1990s was real and not an artifact of sequentially fishing down local populations. In the early 1990s, seasonal fishery and survey data showed distribution changes where there was no fishery, and large tonnage and densities (450 000 t, densities fourfold higher than 1980s levels) appeared in the south concurrent with declines in the north. All fishery, acoustic, and trawl survey indices increased in the south, while the stock declined. Southern-caught cod in the early 1990s exhibited northern characteristics: (*i*) antifreeze production capacities above historical norms and equivalent to those of northern fish, (*ii*) vertebral counts above historic norms and equalling northern counts, and (*iii*) declines in size-at-age to levels associated with northern fish. The cause of the shift is thought to be a combination of abiotic (climate) and biotic (capelin (*Mallotus villosus*)) environmental changes and cumulative long-term fisheries effects on cod behavior. The shifted distributions increased vulnerability to Canadian and foreign fisheries and led to a rapid decline in abundance, both before and after the moratorium on fishing in Canadian waters in 1992. Rebuilding will occur in three steps: environmental restoration, recolonization by adults, and enhanced recruitment across the shelf.

Résumé : Des travaux de recherche sur la morue franche (*Gadus morhua*) réalisés de 1983 à 1994 ont montré que le déplacement apparent des populations de morue vers le sud au début des années 90 était bel et bien réel et non pas un artefact qui serait lié à une diminution progressive des populations locales due à la pêche. Au début des années 90, les données de la pêche saisonnière et des campagnes d'échantillonnage ont montré que des changements se produisaient dans la distribution des morues même en l'absence de pêche, et un fort tonnage et de grandes densités (450 000 t, densités quatre fois supérieures à celles des années 80) ont été observés dans le sud tandis qu'on observait des déclins dans le nord. Tous les indices des pêches, des relevés acoustiques et des échantillonnages au chalut se sont accrus dans le sud tandis que le stock déclinait. Les morues capturées dans le sud au début des années 90 avaient des caractéristiques des morues du nord : (*i*) capacités de production d'antigel supérieures aux moyennes historiques et équivalentes à celles des morues du nord, (*ii*) nombres de vertèbres supérieurs aux moyennes historiques et égalant les nombres observés chez les morues du nord et (*iii*) baisses de la taille selon l'âge à des niveaux comparables à ceux observés chez les morues du nord. On pense que ce déplacement serait dû à une combinaison de changements environnementaux abiotiques (climat) et biotiques (capelan (*Mallotus villosus*)) et aux effets cumulatifs à long terme de la pêche sur le comportement de la morue. Le déplacement des populations vers le sud a accru leur vulnérabilité à l'égard des pêches canadiennes et étrangères et a entraîné un déclin rapide de l'abondance de la morue, tant avant qu'après l'imposition en 1992 du moratoire sur la pêche dans les eaux canadiennes. Pour qu'il y ait reconstitution des populations, il faudra qu'il y ait restauration de l'environnement, recolonisation par des adultes et accroissement du recrutement sur toute la plate-forme.

[Traduit par la Rédaction]

Introduction

Polarized arguments about the effects of fishing and the environment on fish stocks are common in the fisheries literature (for reviews, see Parsons and Seki 1995; Larkin 1996). The recent declines of the "northern" cod (*Gadus morhua*) off northeast Newfoundland and Labrador have rekindled such debate (Mann 1993; Hutchings and Myers 1994). On

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the one hand, environmental conditions and water temperatures approached the severest ever recorded in the late 1980s and early 1990s in Newfoundland waters (Colbourne et al. 1997*a*). By the early 1990s, cod size-at-age and growth were reduced (Bishop et al. 1993; Krohn et al. 1997), recruitment was generally poor (Drinkwater and Mountain 1997), and distribution and migration patterns were changing (Rose 1993; deYoung and Rose 1993). Moreover, other elements of the ecosystem were not stable during this period. For example, capelin (*Mallotus villosus*) declined and retreated southward (Frank et al. 1996), noncommercial species shifted seaward (Gomes et al. 1995), and northern species such as pandalid shrimp (*Pandalus* spp.) and Arctic cod (*Boreogadus saida*) expanded their ranges southward and increased in abundance well beyond previously recorded levels (Drinkwater and Mountain 1997). On the other hand, overfishing has been epidemic since the 1950s and is thought to be the major cause of the declines of many Northwest Atlantic fish stocks (Sinclair and Murawski 1997). There is general agreement that decades of overfishing, beginning with the foreign fisheries of the 1950s and 1960s, and continuing through the 1970s and 1980s primarily with Canadian fisheries, precipitated a severe decline in the northern cod that culminated in the lowest abundance levels known by the early 1990s (deYoung and Rose 1993; Hutchings and Myers 1994). The fisheries could also have contributed, directly or indirectly, to the observed changes in other aspects of the ecosystem (Jennings and Kaiser 1998).

Larkin (1996) suggested that catastrophic stock declines may result from the co-occurrence of detrimental factors, be they fishery or environmental (the inverse could be applied to abrupt increases, such as have recently occurred with shrimp, Arctic cod, and snow crab (*Chionoecetes opilio*) in Newfoundland ecosystems). Larkin's (1996) view recognizes the complexities of interactions in marine environments. For example, climatic or oceanographic factors may affect productivity at different, perhaps longer, time scales than do most fisheries (Brander 1997). As a consequence, the same level of fishing might impact commercial fishes and ecological communities differently under differing environmental conditions. Hutchings (1996) implied that such environmental hypotheses about the northern cod were suspect because they might be attractive to those who assert that the collapse was environmentally driven. However, there should be no abrogation of management responsibility for the success or failure of fisheries implied in such hypotheses (by blaming failures on environmental down-turns). To the contrary, consideration of the environment implies that management has broader responsibilities than traditionally acknowledged, including consideration of "ecosystem" processes and how these may affect both productivity and vulnerability of stocks and communities to fishing.

The northern cod ecosystem historically encompassed a vast area from the northern Labrador Shelf to the Grand Bank (Fig. 1). Stock declines occurred first in the north (NAFO division 2GH, hereafter 2GH; other NAFO divisions cited similarly) in the late 1950s, 1960s, and 1970s and then in the midnorth (2J) in the late 1980s and early 1990s. As the decline proceeded, the relative abundance of cod shifted southward (Atkinson et al. 1997), and age structures collapsed (Bishop et al. 1993). The southern region retained the majority of the extant biomass in the mid-1990s, and in the late 1990s, the southern coastal area enjoyed the greatest stock rebuilding (Lilly et al. 1999).

The declines of northern cod in the 1960s followed massive fisheries on the northern spawning grounds prosecuted primarily by Russian and other then eastern block countries (Travin and Pechenik 1963). Reported catches broached 500 000 t in several years in the mid-1960s (a maximum of >800 000 t reported in one year). Although no direct estimates of cod biomass from that era exist, simulation models suggest an historic biomass in the range of 2.5–3.5 million t (Fahrig et al. 1998). The northern cod is among the least productive of the major cod stocks (Brander 1994). A harvest of approximately 10% of the biomass is likely to be sustainable (approximately half of $F_{0.1}$) and is consistent with traditional total catches of 150–300 000 t·year^{-1} (Harris 1990). Natural fluctuations in productivity may have been substantial as a consequence of the northern climatic range of this stock (Mann 1993; Fahrig et al. 1998). The late 1950s and 1960s were punctuated by warm ocean conditions favorable for growth and perhaps recruitment (Drinkwater and Mountain 1997) and by northerly cod distributions (deYoung and Rose 1993). The extraordinarily high catches of the mid-1960s indicate high levels of abundance and accumulated productivity. However, the northern cod did not sustain the harvests of the 1960s, even in the short term (Harris 1990), and by the early 1970s was in rapid decline. Potential long-term effects of these fisheries on spawning stock distribution and productivity may be significant but are difficult to assess. Nevertheless, deYoung and Rose (1993) presented evidence that cod were still widely distributed in the north, at least to Hamilton Bank, in the aftermath of this debacle and attributed the relatively rapid rebuilding of northern cod in the late 1970s and early 1980s, despite low spawning biomass, to comparatively warm conditions and the maintenance of northerly spawning off southern Labrador.

deYoung and Rose (1993) hypothesized that adult cod shifted their distribution southward in the late 1980s and early 1990s, a view supported by several additional studies (Gomes et al. 1995; Kulka et al. 1996; Atkinson et al. 1997; Warren 1997). Southerly distribution shifts were hypothesized to increase the likelihood of poor recruitment (deYoung and Rose 1993) and, because of the southerly location of the major fisheries, to have contributed to the rapid decline of this stock in the late 1980s and early 1990s (Rose et al. 1994). Alternatively, Hutchings and Myers (1994), reinforced by Hutchings (1996), discounted the shift hypothesis and argued that apparent distribution changes were solely the result of local overfishing, first in the north and then proceeding southward.

The importance of whether real distribution shifts occurred relates not only to a reconstruction of historical events but also to attempts to project and manage the rebuilding of the northern cod. If cod did shift southward under adverse conditions, they might be expected to expand their range northward once conditions improve and hence recolonize the key northern spawning grounds. Under these assumptions, protection of seemingly isolated southern populations may be critical to the rebuilding of the full stock. Alternatively, if no movement occurred in the past, but populations simply collapsed in place (Hutchings and Myers

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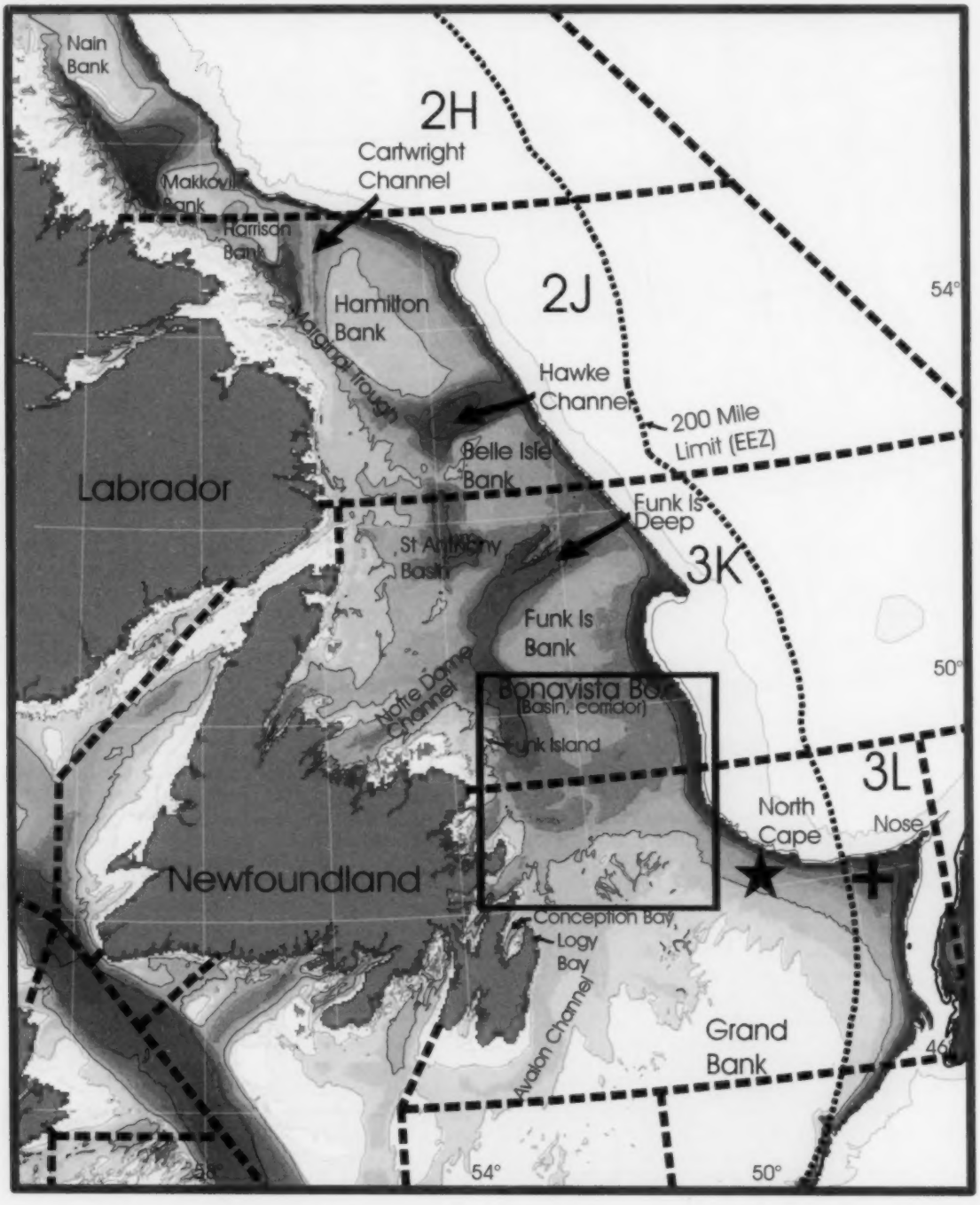
Fig. 1. Newfoundland region and the northern cod ecosystem. The tagging site in May 1991 is indicated by a star, and the location of cod aggregations in the winter and spring of 1993 is indicated by a cross.



1994; Hutchings 1996), a checkerboard of discrete and spatially rigid populations is implied, and there is less reason to protect locally rebuilding populations as they recover.

The shift hypothesis generates several predictions:(*i*) cod distribution changes did not follow fishing activity in the late 1980s and early 1990s (in the extreme case, distributions changed in the absence of a fishery), (*ii*) large numbers of cod were present in the southern ranges beginning in the

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late 1980s and early 1990s as densities abruptly increased above historical levels, (*iii*) availability of cod to the fisheries in the south increased after 1989 (and decreased in the north) independent of declining abundance, and (*iv*) northern fish were present in the southern area in the early 1990s. In addition, the shift hypothesis is consistent with the occurrence of further declines in adult abundance after the moratorium on fishing in the Canadian exclusive economic zone (EEZ) in 1992 because the southern fishery outside the Canadian EEZ was not under moratorium. The alternative hypothesis, of local depletion, predicts fish distribution changes that follow fishery concentrations, no buildup of cod in the southern range after 1989, no increase in availability in southern waters, and no northern fish in the southern range in the early 1990s. Declines in abundance after 1992 are inconsistent with the local depletion hypothesis, unless some other mortality factor is considered.

In this paper, we examine the predictions of the shift hypothesis, address the question of what happened to the northern cod in the late 1980s and 1990s, and discuss our findings as they relate to stock rebuilding. We present data from sea and laboratory research on cod distribution, migration, physiology, and oceanographic conditions, integrated with published documentation from Department of Fisheries and Oceans (DFO) surveys and the inshore fisheries. Our "view from the ocean" is built therefore upon direct observation and experimentation conducted at sea during the most recent decline of the northern cod.

Methods

DFO has conducted bottom-trawl surveys using a stratified random design since 1978 (Bishop et al. 1993). Most fishing sets were accompanied by a near-bottom temperature measurement. The methods underpinning these surveys are well known and described (Doubleday and Rivard 1981).

Acoustic searches for cod were conducted annually in May to early July from 1983 to 1994 in the area bounded by 48°00.0′ and 50°00.0′N and 050°00.0′ and 053°00.0′W (this region approximates the southerly cross-shelf migration route for this stock, called the Bonavista corridor, after Rose (1993)) (Fig. 1). From 1983 until 1989, the chief purpose of the work was to tag cod (echosounders were used to locate cod aggregations), whereas from 1990 until 1992, tagging and more formal acoustic surveying were undertaken together. In 1993 and 1994, only acoustic surveying was done. The RV *Gadus Atlantica*, using towed 49- and 38-kHz transducers, undertook the acoustic surveying in all years and used an Engels 145 otter trawl to capture cod. Some additional tagging was conducted from the RV *Lady Hammond* in 1991 and the RV *Petrel V* in 1992. In all years, the same strategy was used: echosounders were used to locate cod aggregations, and then, short-duration (5–15 min) fishing sets were undertaken on those aggregations (catches >90% cod in all cases). In 1993 and 1994, no tagging was undertaken as a consequence of the moratorium on fishing (July 1992), but fishing sets were conducted in the same fashion.

The echosounding record was not uniform from 1983 to 1994, and uncertainties about calibration in the early years disallowed the use of standard acoustic integration techniques (MacLennan and Simmonds 1992). However, scientific echosounders were used (Simrad EK400, Biosonics 102), and good-quality echograms existed for all years. The distribution of cod was in most cases amenable to counting techniques. A method was developed to quantify these signals as to relative cod density based on a visual counting scheme. First, all cod aggregations supported by fishing sets were identified on the echograms from the decade of data. For each aggregation identified, a minimum of five randomly placed "sampling windows" were placed over the echogram, and all single echoes within the window were counted. If echoes overlapped, counts were estimated (comparisons between counts and integrated densities in the fully calibrated data of the later years indicated that counts underestimated biomass, especially at higher densities). To normalize sampling volumes, a variation of the "duration in beam" technique was used (Crittenden et al. 1988). The width of a minimum of five fully formed single fish echoes was measured to the nearest 0.1 mm on the echogram (this width will be a function of vessel speed, depth, and fish size). All counted densities were then "normalized" to the mean width of the single fish trace. This technique provided a measure of the relative local densities of cod that was for the most part free from calibration and system related biases and could be used to compare the 1983–1994 data.

Acoustic data from 1990 to 1994 were collected with scientific echosounders calibrated according to standard methods with a 38-mm tungsten carbide standard target (Foote et al. 1987). Migrating cod aggregations are near monospecific (Rose 1993). Species identifications were made on the basis of target strength and echogram interpretations supported by directed fishing. Data were integrated over either 100 or 1000 m, scaled using a target strength to mean length model for cod (Rose and Porter 1996), and represented as fish per square metre based on 1-km transect segments. Segments from each surveyed aggregation were then randomly sampled at a decreasing rate until autocorrelation was not significant ($P > 0.05$). Mean abundance and SE were estimated from Monte Carlo sampling of the density data at that rate with n being the number of samples at which autocorrelation was not significant. Confidence intervals thus include sampling variance only. Error from detectability (Lawson and Rose 1999) and target strength is thought to be minimal because cod were distributed well off the bottom (to 150 m) during most of this work (see Rose 1993), and target strength experiments were conducted as a part of this research (Rose and Porter 1996).

Fishery data have been collected through an observer program on Canadian fishing vessels since 1980 (Kulka and Firth 1987). Prior to 1987, approximately 20% of vessel-days were monitored. Since 1987, there has been 90–100% coverage in the winter fishery. The full data set used here includes a total of 275 488 otter trawl sets made during the winter–spring fishery (January–July of 1981–1992) in 2J, 3K, and 3L (the northern cod). The subset of the data for the Bonavista corridor comprises a total of 65 702 sets made from January to July of 1981–1992. Each set was monitored as to location, duration, gear type, depth, and catch by species. Catch data were standardized by vessel, gear type, duration, and month prior to analyses (for full details, see Kulka et al. 1996).

In June 1994, acoustically identified cod aggregations were sampled using otter trawls at three sites within the historic range of the northern cod (Hawke Channel, Notre Dame Channel, and the Bonavista Basin) and from the north cape of the Grand Bank (Fig. 1). Vertebral columns from randomly selected fish from the catch from each aggregation were frozen at sea, subsequently thawed and X-rayed, and vertebral numbers counted. We followed the method of including the last urostylar vertebrae (Templeman 1981). Blood samples for genetic analyses were taken from 50 fish from each aggregation. The results of subsequent analyses have been reported by Ruzzante et al. (1998).

Adult cod (45+ cm) were monitored for antifreeze production over 14 winters between 1980 and 1994. Cod were collected annually from Conception Bay and south of Logy Bay on the eastern Avalon during the summer and fall (July–September) by commercial fishers prior to the moratorium in 1992 and by scuba divers from the Ocean Sciences Centre, Memorial University of Newfoundland, thereafter. Each winter, only cod caught in the preced-

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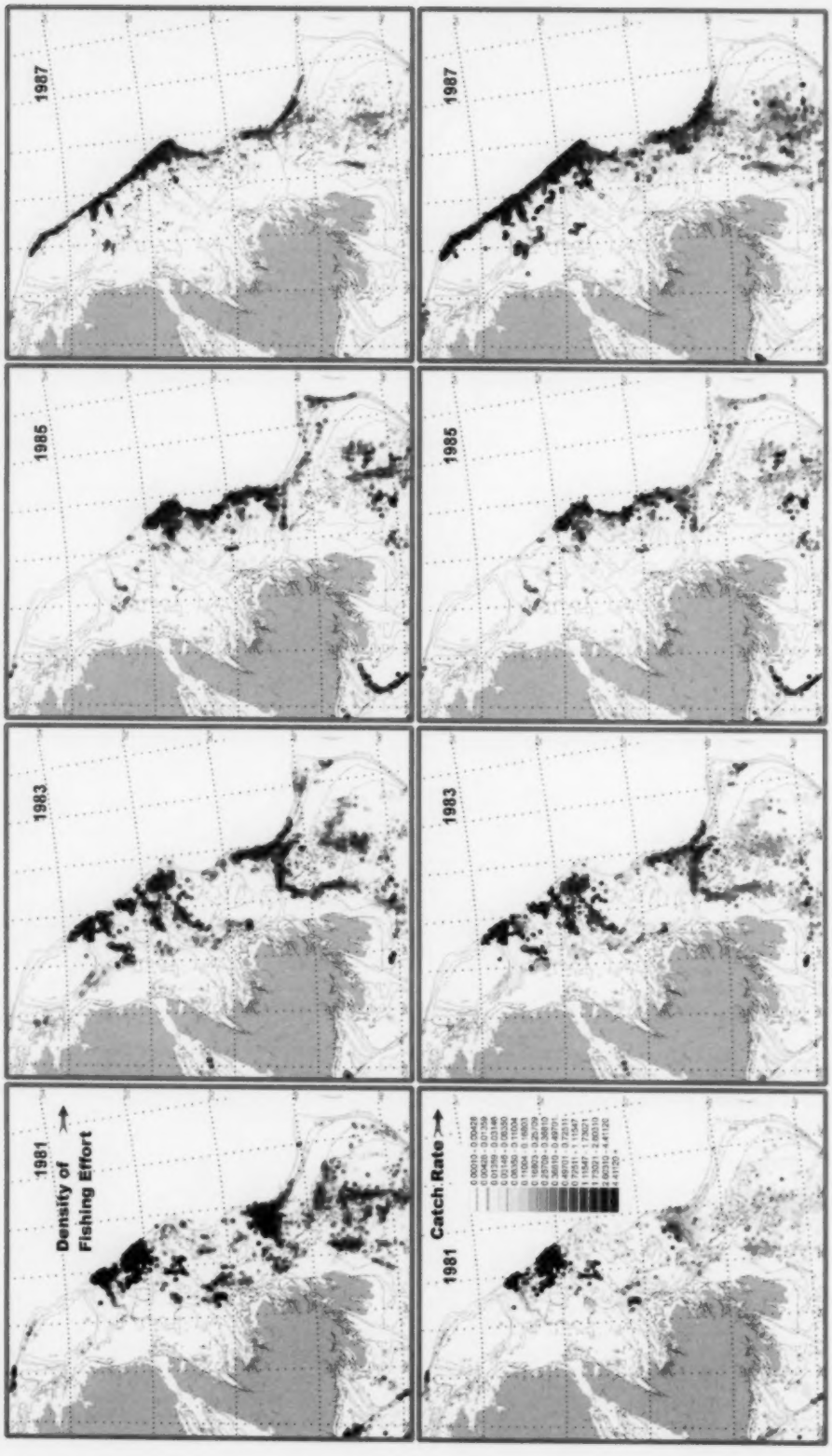

Fig. 2. Density of fishing effort and catch rate ($t \cdot h^{-1}$) from the offshore winter trawler fishery for northern cod for 1981, 1983, 1985, and 1987.

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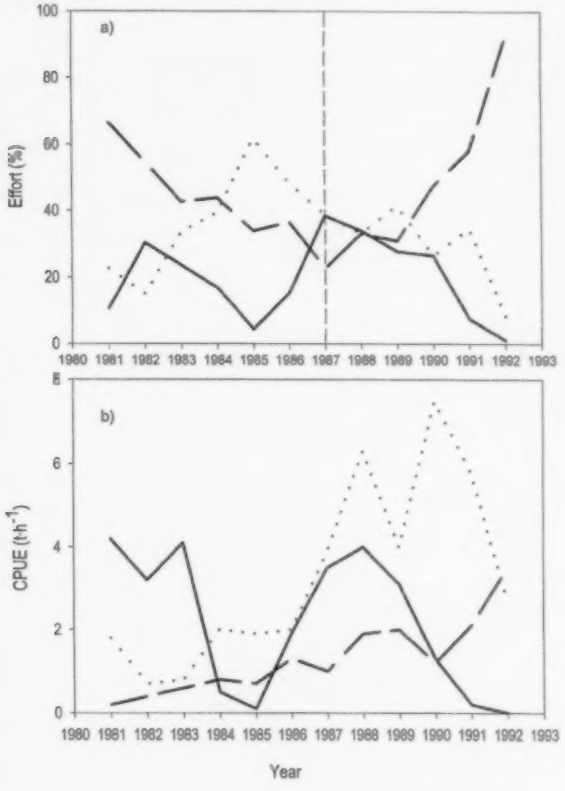
Fig. 3. (*a*) Percentage of fishing effort and (*b*) mean CPUE for the offshore winter trawler fishery for northern cod in 2J (solid line), 3K (dotted line), and 3L (dashed line) from 1981 to 1992. The vertical dashed line represents introduction of the one third rule that attempted to disperse effort evenly over the three regions.



ing summer were monitored for antifreeze production. Cod were held in experimental tanks (either a 40 000-L raceway or one of three cylindrical tanks of approximately 2000–2700 L) and were exposed to seasonally ambient conditions of temperature and photoperiod throughout the experiments. Tanks were supplied with seawater pumped continuously from approximately 10 m depth in Logy Bay (Fig. 1). Water temperatures were recorded several times a week while the cod were under investigation. Cod were fed chopped capelin twice weekly throughout, and each experiment was terminated at some point during the summer.

Blood samples were taken at approximately monthly intervals over the winter cycle (December–June in most years). Blood samples (1–2 mL) were withdrawn from a caudal blood vessel using 3-cm^3 syringes with 21- or 23-gauge needles. Sampling was quick (<1 min) and no anaesthetic was used. Samples were injected into Vacutainers containing sodium heparin (Becton Dickinson) and held on ice for a short period prior to centrifuging at 4000 × *g* for 10 min. The plasma was then removed from the cells and stored frozen at –20°C until analysed for antifreeze activity.

In common with many teleost species inhabiting high latitudes, Atlantic cod produce antifreeze glycoproteins (AFGPs) that interact with ice crystals in a noncolligative manner to inhibit ice crystal growth, lower the plasma freezing temperature, and increase the fishes' ability to withstand exposure to extreme low temperatures (Fletcher et al. 1987; Davies and Hew 1990). The freeze-depressing action of AFGP is between 200 and 300 times greater than would be predicted based on the number of antifreeze molecules in solution (i.e., colligative properties) alone (DeVries 1988). However, since the AFGPs have no influence on the melting point (other than the expected colligative effects), a solution containing antifreeze will have a lower freezing point than melting point. This difference between the freezing and melting points is termed thermal hysteresis (TH). TH is directly proportional to the concentration of antifreeze in solution and is used as a direct measurement of antifreeze activity.

Analysis of antifreeze activity within each plasma sample was carried out using a nanolitre osmometer (Clifton Technical Physics, New York) according to the method of Kao et al. (1986) in which a small volume of plasma containing a single ice crystal is cooled and heated in a controlled and quantifiable manner. By microscopic observation of the resultant growth and shrinkage of the ice crystal, the freezing and melting points, and hence TH, of the plasma sample can be determined. Standard solutions used to calibrate the osmometer commonly give TH readings of approximately 0.07°C; thus, such values indicate that antifreeze is absent from the plasma.

Results

Did cod distribution changes follow high levels of fishing?

Offshore trawler fishing effort during the 1980s was widely distributed over the stock area from 2J to 3L, although areas of concentration varied from year to year (Fig. 2). Mean annual effort was highest in the south and lowest in the north in the 1980s and until the moratorium in mid-1992 (Kruskal–Wallis test, $P < 0.05$; mean number of sets per year were 4034, 3503, and 2452 in 3L, 3K, and 2J, respectively, excluding 1992 when this fishery was truncated in February) (Fig. 3*a*). Effort was also more variable among years to the north, especially in 2J (coefficients of variation for 2J, 3K, and 3L were 0.9, 0.5, and 0.4, respectively). A management measure introduced in 1987 resulted in a more even dispersion of effort over the full stock range for a few years (the so-called one third rule) (Fig. 3*a*). However, from 1990 to 1992, effort again shifted to 3L. In contrast with effort, catch-per-unit-effort (CPUE) in 3L was the lowest of the regions in the early 1980s, while CPUE was much higher in 2J (Fig. 3*b*). In 1984 and 1985, CPUE in 2J declined but rebounded to levels comparable with those of the early 1980s from 1986 to 1989. From the mid-1980s until 1992, peak CPUE shifted southward, with maximums in 1988 in 2J, 1990 in 3K, and 1992 in 3L. CPUE remained high in 3L (the highest on record for this region) until the moratorium in 1992. Annual CPUE and effort were not significantly correlated in any region, but correlation was stronger in the north (*P* values for 2J, 3K, and 3L were 0.06, 0.07, and 0.89, respectively).

The period from 1988 to 1994 encompasses the most recent decline of the northern cod and the proposed distribution shift (Fig. 4). The fall trawl survey, winter fishing CPUE, and spring acoustic survey data all show declines in the spatial extent of high densities of cod, and southward shifts, during this period. However, in the fall of 1988 and the winter of 1989, cod were still distributed over the full stock range from north to south and across the shelf.

An increasing concentration of fish in the southern zone (southern 3K and northern 3L) is initially evident in the fall survey of 1989 and the following winter fishery of 1990

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Fig. 4. Sequential data from the fall DFO research vessel survey (kg·tow^{-1}), the winter fishery (t·h^{-1}), and the spring acoustic/trawl survey (fish·m^{-2}) from 1989 to 1994.

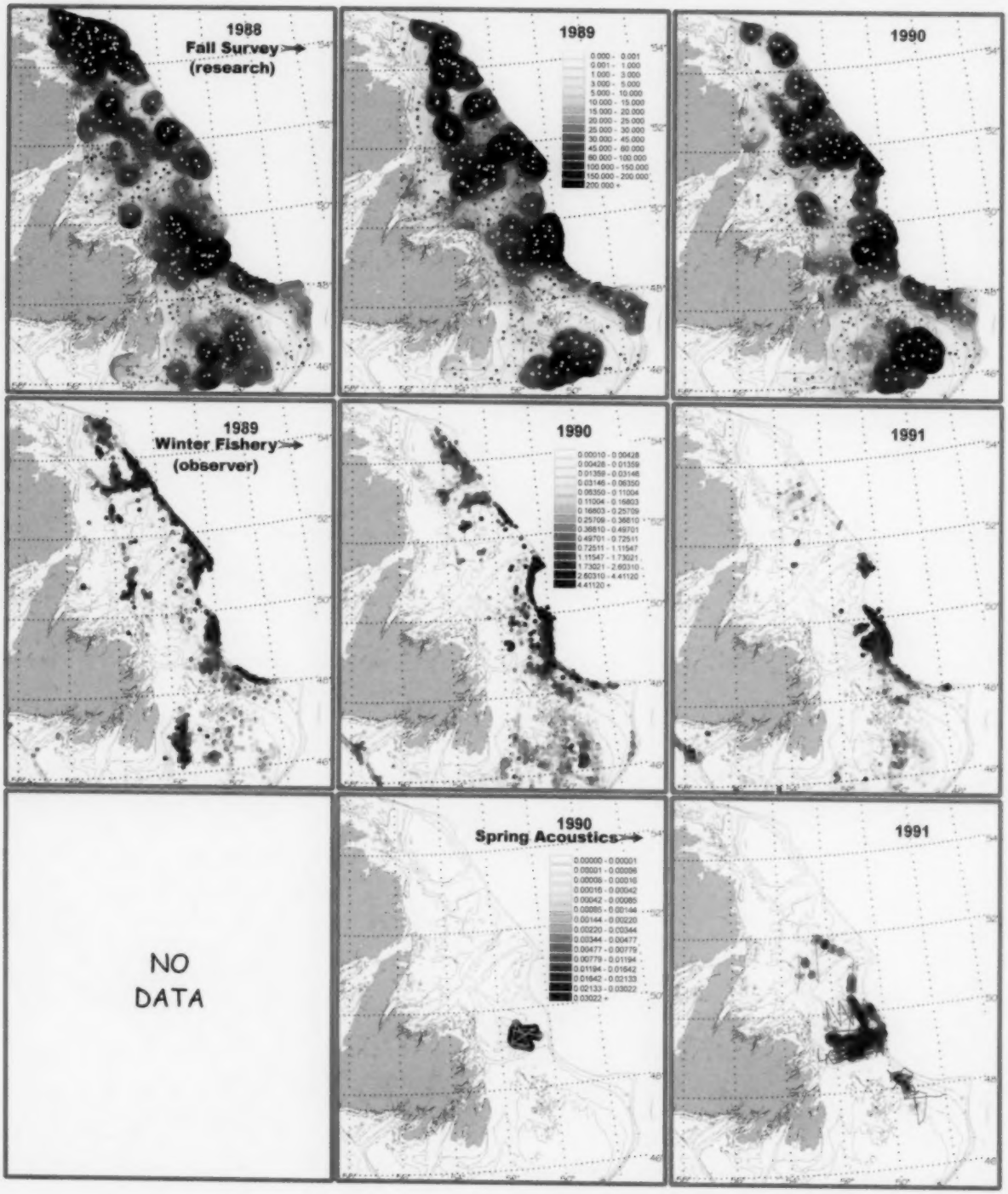

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Fig. 4 (*concluded*).

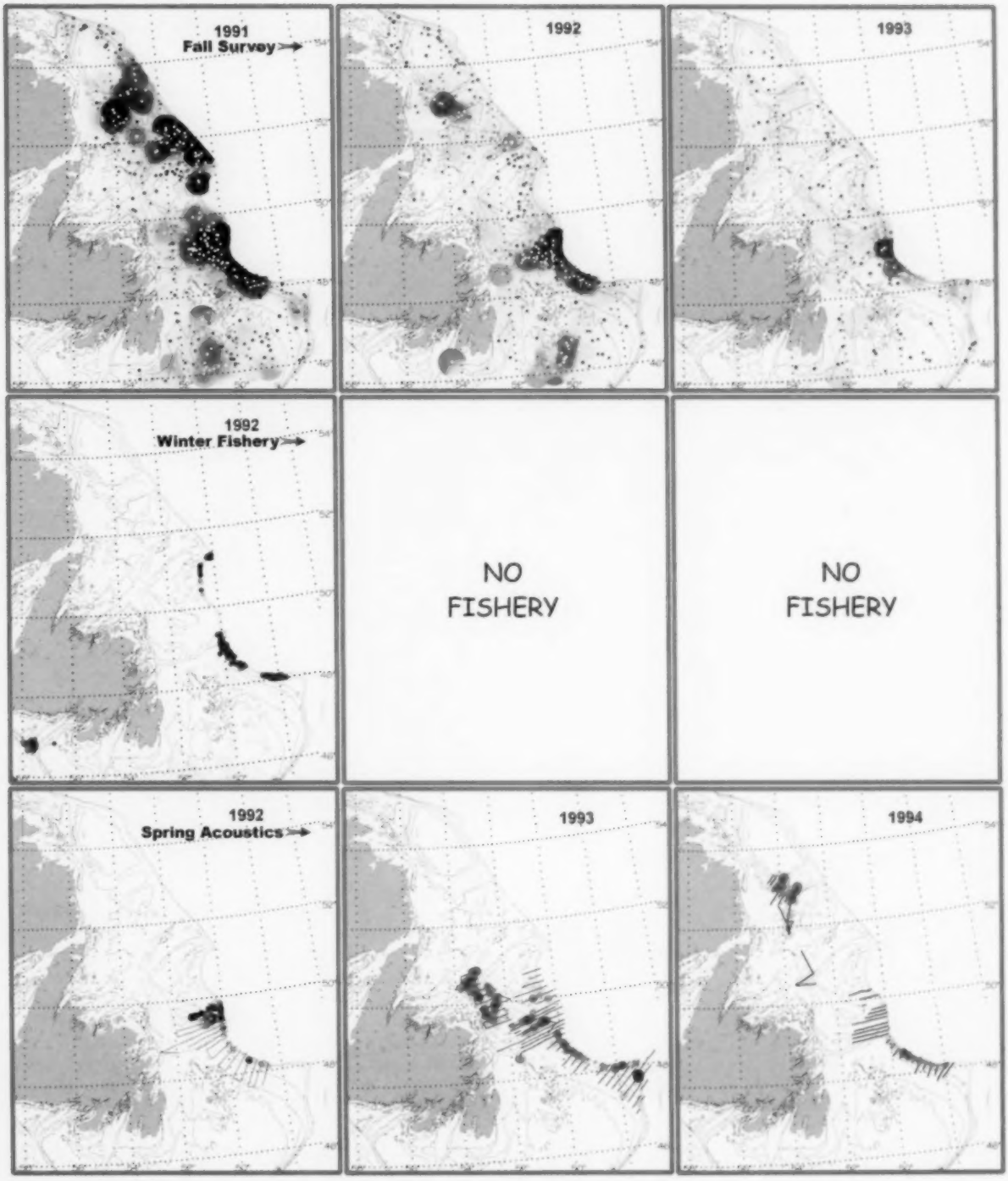

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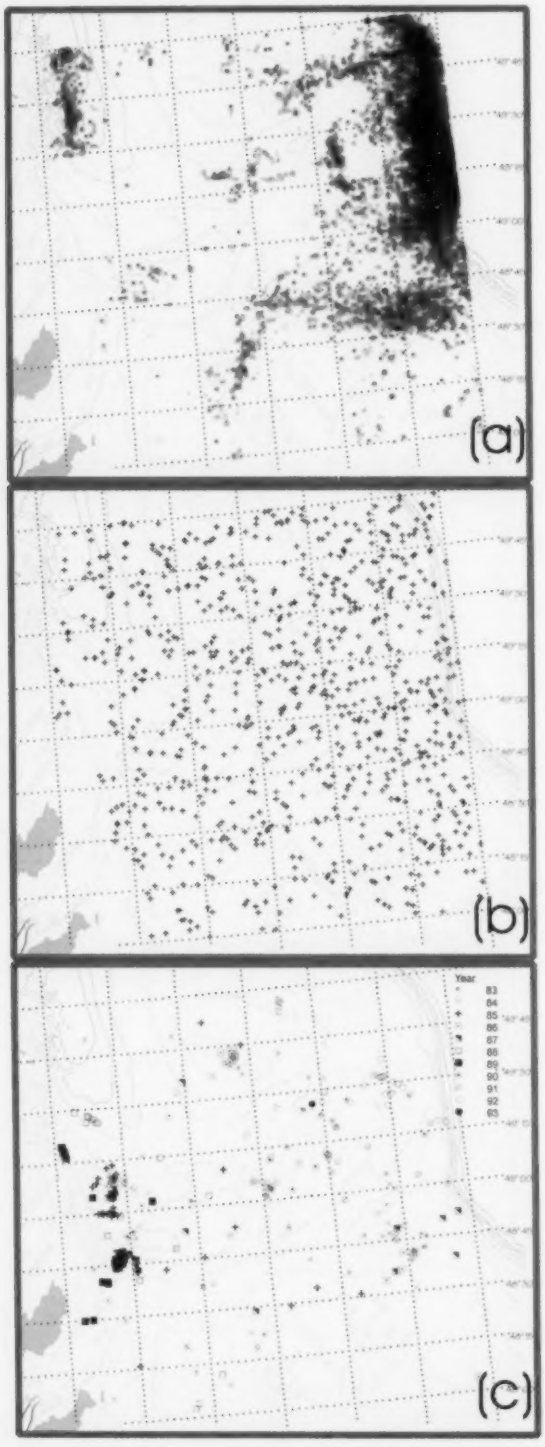
Fig. 5. Bonavista corridor study area (*a*) winter fishery trawl sites, (*b*) fall trawl survey fishing sites, and (*c*) spring research fishing sites for 1983–1993. Only the spring data are shown by year (no spatial patterns exist in the other series).



(Fig. 4). CPUE in 2J declined precipitously in winter 1990 but reached its highest value on record further south in 3K (Fig. 3*b*). Spring surveys in 1990 confirmed the existence of very high densities in southern 3K and northern 3L (Fig. 4). The fall 1990 survey showed fewer fish in the north relative to earlier surveys, but a broad distribution from north to south was still evident. However, in the following few months, the 1991 winter fishery failed completely in the north, while CPUE remained elevated in the south. The 1991 spring survey again confirmed the presence of high densities of fish in the south. The 1991 fall survey located much reduced densities but still a broad distribution from north to south except in northern 2J. The 1992 winter fishery took virtually no fish from the north and, despite very high CPUE in the south, was closed in February 1992. The 1992 spring survey showed very high densities of cod in the south over a reduced area. The 1992 fall survey indicated a further decline in the north and one area of concentration in the south (the same area of concentration earlier indicated by the spring surveys and winter fisheries). There was no cod fishery within the Canadian EEZ in 1993. The 1993 spring survey found high densities of cod well to the southeast and outside the EEZ. No concentrations were found inside the EEZ. The 1993 fall survey indicated additional declines in the north and south. The 1994 spring survey found further declines over the southern range, and virtually no cod were located within the main migration corridor in use from 1990 to 1992. Distributions of cod on the Grand Banks (46–47.5°N) also declined beginning in the fall of 1991.

Did fish abundance and density increase in the southern range after 1989?

From 1983 to 1994, commercial and survey fishing sets, and sets directed at cod aggregations during acoustic and tagging experiments, were widely distributed in the Bonavista corridor study area (Fig. 5). In the acoustic-tagging data, sets and aggregations tended to be concentrated within years. Mean standardized annual catch rates ranged from 100 to 500 kg·0.5 h^{-1} and did not differ from 1983 to 1989 (Duncan's multiple range test, $P > 0.05$) (Fig. 6*a*). However, in 1990, catch rates quadrupled and remained significantly above historical levels through 1991 and 1992 ($P < 0.05$). In 1993 and 1994 (not shown), catch rates fell to levels below those of 1983–1989.

Mean acoustic densities of the same cod aggregations in the Bonavista corridor showed a pattern similar to the catch rates with respect to the increases after 1989 and the decline in 1993, but the magnitude of the variations was greater (up to an order of magnitude) (Fig. 6*b*). In addition, densities were higher from 1983 to 1985 than during the years 1986–1989 (Duncan's multiple range test, $P < 0.05$).

Mean annual catch rates from the DFO fall survey within the Bonavista study area were similar through the 1980s but increased five- to sixfold in 1990 (Fig. 6*c*). Catch rates declined to levels similar to those of the 1980s after 1990. Commercial CPUE became elevated in 1990 (there is some evidence of an earlier trend), peaked in the winter of 1991, and remained high in 1992 (Fig. 6*d*).

Acoustic biomass estimates of cod in June in the Bonavista corridor declined from approximately 450 000 t in 1990 to 100 000 t in 1992 (Table 1). After 1992, both distri-

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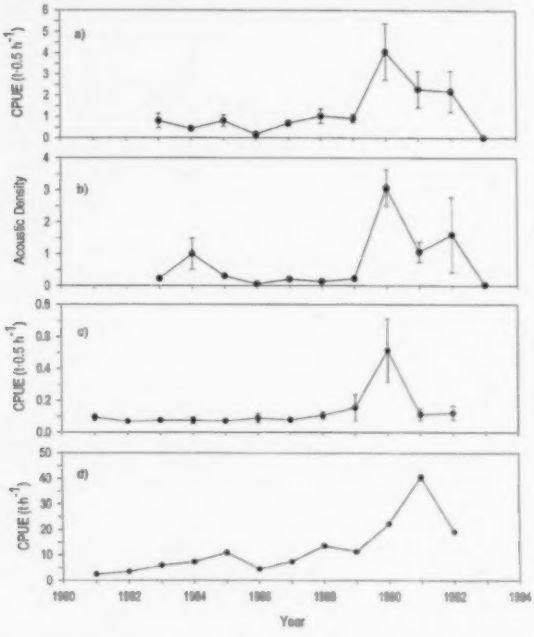
Fig. 6. (*a*) CPUE from directed fishing sets during spring research (standardized to a 30-min tow), (*b*) acoustic densities for the aggregation fishing during the spring research, (*c*) CPUE from the fall trawl surveys, and (*d*) CPUE from the winter fishery in the Bonavista corridor from 1981 to 1993 (mean and 95% CI).



bution and abundance continued to decline (Fig. 4; Table 1). In 1993, postspawning adults could be located only to the southeast of the Bonavista corridor and north cape areas on the nose of the Grand Bank outside the Canadian EEZ (Fig. 1).

In May 1991, a large cod aggregation was tracked for 5 days as it moved eastward along the north cape of the Grand Bank toward the EEZ limit. After the tracking experiment, 2200 cod from this aggregation were tagged in relatively shallow and cool waters (approximately 200 m, 0°C) and released (Fig. 1). Only three tags have ever been returned (one from a lone Canadian vessel fishing nearby a few days after tagging). By contrast, tagging experiments conducted a few weeks later but further to the north and in deeper and warmer waters (>350m, 3–4°C), where fish are known to have ventured to inshore Newfoundland (Rose 1993), have had tag returns of 15–20% (see Taggart et al. 1995).

In 1992, the extant northern cod were concentrated at the offshore margins of the Bonavista corridor (Fig. 4). However, in February 1993, a DFO acoustic survey failed to locate any fish in this corridor. The same survey reported the location of a large cod concentration (no firm estimate) approximately 50 km outside the Canadian EEZ on the nose of the Grand Bank (J. Baird, Northwest Atlantic Fisheries Centre, St. John's, Nfld., personal communication) (Fig. 1). In June 1993, a more comprehensive survey of this region located a single aggregation of adult cod spanning 8 km (acoustic estimate 20 000 t) approximately 50 km from the winter location and 40 km outside the EEZ. By 1994, few

Table 1. Acoustic biomass estimates for cod in the Bonavista corridor in June of 1990–1994.

	Biomass (t)	SE
1990	450 000	60 000
1991	300 000	35 000
1992	100 000	10 000
1993	20 000	3 000
1994	2 000	1 000

Note: SE values are based on transect sampling error only.

adult or juvenile cod could be found anywhere on the northeast shelf or northern Grand Bank inside or outside the EEZ by trawl or acoustic surveys (Fig. 4).

In the inshore trap fisheries, total catch in the southern range (3L) in 1990 was the highest on record for the period 1975 until the moratorium in 1992 (Fig. 7*a*). Availability increased three- to four-fold in 1990 and 1991 over the values recorded in the 1980s (Fig. 7*b*). In contrast, in the northern range of the stock (2J), both catches and availability declined steeply after 1989.

Were "northern" fish present in the southern ranges?

From 1980 to 1991, a consistent pattern of antifreeze production in relation to temperature and time of the year was observed in adult cod caught during the previous summer off the Avalon Peninsula (Fig. 8*a*). In all years, antifreeze was absent from the plasma in late summer – fall. As water temperatures declined to 0°C and below (end of December – January), production of antifreeze was initiated so that small but measurable quantities appeared in the plasma by early January. Levels rose throughout the winter, reaching mean peak values of TH towards the end of March. Water temperatures generally rose above 0°C during April, and, coincident with this, antifreeze began to be lost from the plasma. By June–July, summer plasma freezing points of approximately –0.7°C were again the norm and TH readings were again at or below 0.07°C.

During this period (1980–1991), the highest mean levels of TH (mean TH ± 1 SE) were observed in March of 1982 (0.462 ± 0.024°C, n = 13), 1989 (0.462 ± 0.049°C, n = 10), and 1991 (0.461 ± 0.024°C, n = 10). Between 1980 and 1991, only three fish out of a total of 218 sampled during the months of peak antifreeze levels (March and April) developed antifreeze concentrations giving TH values >0.6°C (Table 2). These three fish were all caught in the summer of 1988 and developed their peak TH levels in March 1989.

During the winter of 1991–1992, cod caught in the summer of 1991 exhibited a pattern of antifreeze production unlike any previously observed (Fig. 8*b*). Production was initiated considerably earlier in the year at higher ambient temperatures. By the beginning of January, antifreeze levels were already high, at the time when antifreeze would typically be just appearing in the plasma. By late January, a mean TH of 0.432 ± 0.044°C was recorded, and levels continued to rise to a peak of 0.798 ± 0.1°C by late April 1992. This was 73% higher than the highest mean level recorded over the preceding 10 years. As in previous years, levels de-

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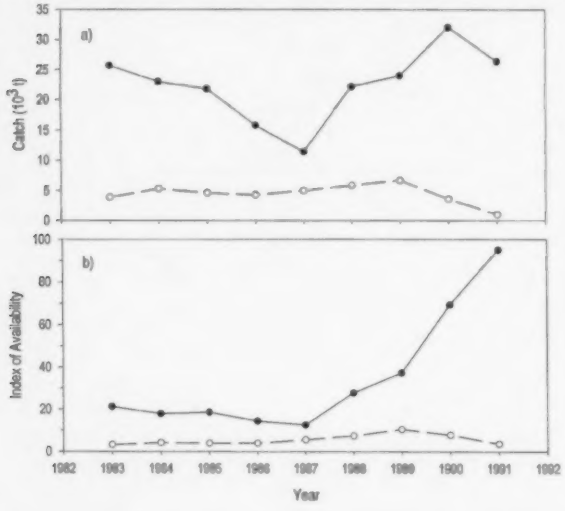
Fig. 7. (*a*) Catches of the trap fisheries in 3L (solid circles) and 2J (open circles) and (*b*) index of the overall availability of fish to the trap fisheries in 3L and 2J (data from Bishop et al. 1993).



clined after April and antifreeze was gone from the plasma by July.

In 1992–1993, 1993–1994, and 1994–1995, cod caught inshore during the summer and held over winter also exhibited patterns of antifreeze production elevated over those of the 1980s (Fig. 8*b*). Of the 67 fish sampled during March and April between 1992 and 1995, 40 fish (60%) developed TH levels >0.6°C; 19.4% of these fish had TH levels higher than any that had been recorded between 1980 (when these studies began) and 1991 (Table 2).

The mean value of all TH data for March of all years collected prior to the shift in 1992 was compared with all the postshift March data using Student's *t* test. Values for April were treated in the same manner. Preshift March mean TH ± 1 SE was 0.38 ± 0.011°C (n = 107). Postshift March TH was 0.63 ± 0.022°C (n = 42). Preshift April TH was 0.32 ± 0.01°C (n = 111). Postshift April TH was 0.70 ± 0.043°C (n = 25). The March postshift mean TH was 64.5% higher than its preshift counterpart, while the April postshift mean was more than double that of the mean of April measurements taken prior to 1992. The differences between the pre- and post-shift values were highly significant for both months ($P < 0.001$)

Water temperatures in the holding tanks were monitored several times a week from 1986 onwards during these studies (Fig. 8*c*). Since AFGP production in adult cod is triggered primarily by low water temperature (Fletcher et al. 1987), it was important to have a clear picture of the thermal conditions to which the cod were exposed during the course of these experiments. The two coldest winters (1988–1989 and 1990–1991) fell prior to the antifreeze shift. Ambient temperatures and antifreeze production capacity were not correlated either before or after 1991 ($P > 0.05$).

Vertebral numbers for the 1990 year-class did not differ among four discrete cod aggregations located in the Hawke Channel, the St. Anthony Basin, the Notre Dame Channel, and the north cape of the Grand Bank in June 1994 (Fig. 1; Table 3). The mean for each group did not differ significantly from vertebral numbers reported by Templeman (1981) for the northeast Newfoundland and Labrador Shelf in the years 1947–1971 or from historical Russian data from

Fig. 8. Plasma TH levels (mean ± 1 SE) for cod captured on the east coast of Newfoundland (Avalon Peninsula) during summer (except 1989), held over the winter subsequent to capture, and monitored for antifreeze production. (*a*) 1980–1990, with 1980–1981 (solid circles), 1981–1982 (open circles), 1982–1983 (solid inverted triangles), 1983–1984 (open inverted triangles), 1984–1985 (solid squares), 1985–1986 (open squares), 1986–1987 (solid diamonds), 1987–1988 (open diamonds), 1988–1989 (solid triangles), 1990–1991 (open triangles), and the curve representing a least squares best-fit model (four terms: $R^2 = 0.8$, n = 631); (*b*) 1991–1994 inclusive (n = 272), with 1991–1992 (solid circles), 1992–1993 (open circles), 1993–1994 (solid inverted triangles), 1994–1995 (open inverted triangles), and the model from Fig. 8*a*); (*c*) seawater temperatures in tanks where antifreeze experiments were conducted from 1986 to 1995, with 1986–1987 (open circles), 1987–1988 (open squares), 1988–1989 (open triangles), 1989–1990 (open diamonds), 1990–1991 (open hexagons), 1991–1992 (solid circles), 1992–1993 (solid squares), 1993–1994 (solid triangles), and 1994–1995 (solid diamonds). Temperatures are given as monthly means calculated from measurements taken at least four times per week throughout the study period.

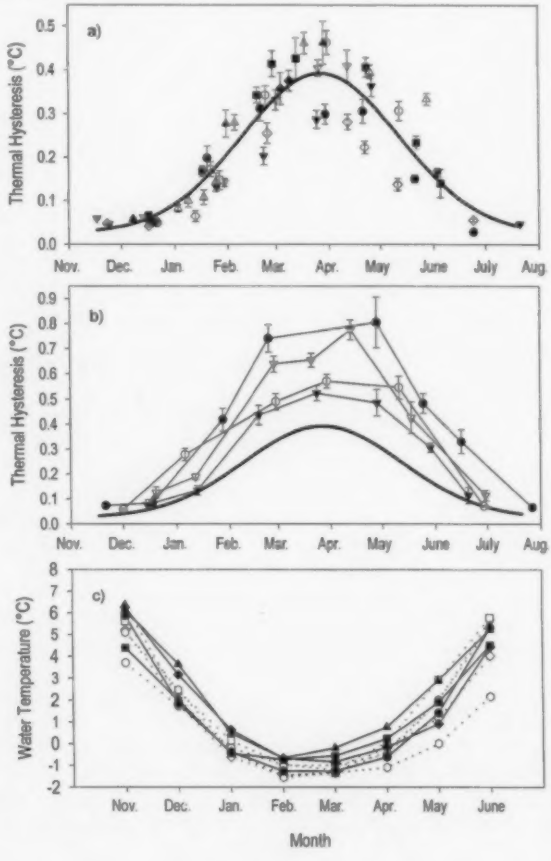


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Table 2. Distribution (%) of TH values (°C, midpoints of ranges) in adult cod (>45 cm) during the months of peak antifreeze levels (March and April) in years prior to (1981–1991) and after (1992–1995) the shift in antifreeze production capacity.

	TH						
	<0.45	0.55	0.65	0.75	0.85	0.95	>1
1981–1991							
March (n = 107)	84	13	2	1			
April (n = 111)	95	5					
1992–1995							
March (n = 42)	17	26	41	7	5	2	2
April (n = 25)	24	12	4	24	20	8	8

Table 3. Mean vertebral counts (SE in parentheses) from cod from four regions on the northeast Newfoundland and Labrador Shelf (Hawke Channel, St. Anthony Basin, and the Notre Dame Channel) and from the north cape of the Grand Bank).

	1994		1961–1971		1947–1960		1960–1961	
	n	Mean	n	Mean	n	Mean	n	Mean
Hawke Channel	41	55.15 (0.16)	613	55.19 (0.09)	300	55.40 (0.08)	298	55.31 (0.06)
St. Anthony Basin	46	55.04 (0.16)	480	55.30 (0.08)	325	55.34 (0.09)		
Notre Dame Channel	40	55.02 (0.12)	105	55.24 (0.11)	226	55.38 (0.08)		
North cape	62	55.02 (0.12)	1180	54.80 (0.03)	590	54.75 (0.10)	258	54.40 (0.07)

Note: Data from 1994 are from fish of total length <35 cm (aged as 1990 year-class). A random selection of commercial and research vessel captured cod were sampled in 1961–1971 and 1947–1960 (data from Templeman 1981). The 1960–1961 data are from Postolakii (1963). Duncan's multiple range test indicated that the historical data from the north cape formed one group and the rest of the data, including the 1994 north cape fish, formed another ($P < 0.05$).

the same area. However, the mean number of vertebrae from the north cape site was significantly higher than reported historically for that area and was similar to that of more northerly located cod (Table 3).

Weight-at-age of cod aged 5–7 years, which comprised over 90% of the migrating fish during the late 1980s and early 1990s, declined in the southern area (3L and 3K) in the early 1990s to levels similar to that of cod from the northern area (2J) in the 1980s (Fig. 9). Cod of age 8 in 3L also declined in weight but not to the levels in 2J. Cod of ages 5 and 6 in 2J declined in weight in the early 1990s, but cod of ages 7 and 8 did not.

What happened in the environment during these changes?

The period of decline of the northern cod beginning in the 1960s has been accompanied by substantial changes and variability in the environment of the Northwest Atlantic (Fig. 10). Vertically averaged temperature, from a station on the inner shelf, shows a decline of greater than 1°C from the late 1960s to the early 1990s (Fig. 10*c*). The period from the mid-1980s to mid-1990s was particularly cold, with the early 1990s the coldest in the 60-year series. The early 1990s featured heavy and prolonged ice cover and low salinities (Figs. 10*b* and 10*d*). Most of the variance associated with the intraannual changes in temperature, wind stress, and sea ice cover can be related to large-scale pressure differences over the North Atlantic. The North Atlantic Oscillation index (NAO) (Fig. 10*a*), which is the pressure difference between the Azores and Iceland, strongly influences interannual variability in the ocean and the atmosphere over the entire North Atlantic (Dickson et al. 1996). It is also likely that there is a feedback between the ocean and the atmosphere that regulates, or at least influences, the periodicity of the NAO, since longer time scale atmospheric phenomena are typically connected to the longer "memory" of the ocean. The NAO reached its highest peak in the 60-year time series in 1989 and 1990 and remained high until 1996.

Discussion

Our work supports the predictions of the shift hypothesis and indicates that cod moved southward on the northeast Newfoundland Shelf in the late 1980s and early 1990s. Cod became aggregated within a small area on the north cape of the Grand Bank and in the Bonavista corridor by the early 1990s. While many of these fish likely came from the north (deYoung and Rose 1993), as evidenced by their smaller size-at-age, enhanced antifreeze production capacity, and higher vertebral count, some may have originated from northern areas of the Grand Bank. These findings are contrary to the hypothesis that no important large-scale changes in distribution or movement patterns occurred in the late 1980s and early 1990s, other than those caused by local overfishing (Hutchings and Myers 1994; Hutchings 1996; Myers et al. 1996). *These conclusions do not discount the importance of overfishing to stock decline.* In contrast, our findings tend to highlight how overfishing could have occurred in such a highly studied, regulated, and managed fishery and how the best-intended management can go awry if ecosystem considerations are not taken into account. Southerly shifting concentrations led to increased vulnerability to overfishing, both legal and illegal, both Canadian and foreign, and resulted in a rapid decline of this stock (Rose and Kulka 1999).

Our data show at least two large-scale changes in cod distribution during the late 1980s and 1990s that were not

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Fig. 9. Mean size-at-age of northern cod of ages 5–8 (*a–d*, respectively) for 1985–1992 from the DFO fall survey in 3L (solid line) and 3K (dotted line), and mean size-at-age during the 1980s in 2J (square) (data from Bishop et al. 1993).

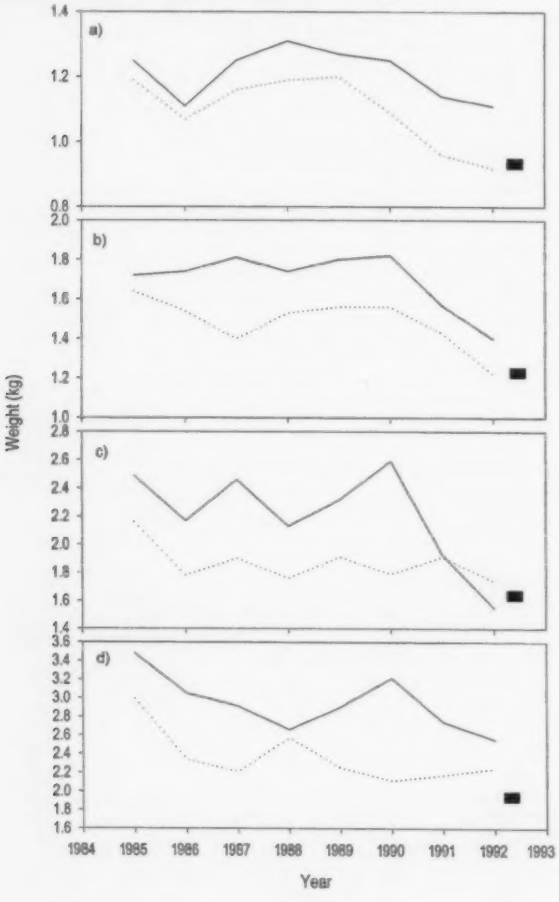
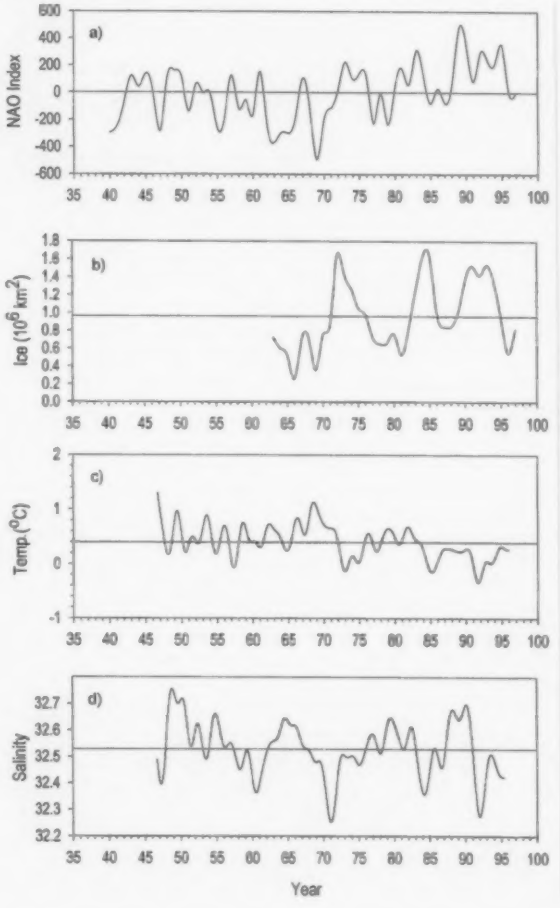


Fig. 10. (*a*) Annual average NAO, (*b*) ice cover averaged annually for the Newfoundland and Labrador Shelf, (*c*) temperature, and (*d*) salinity for 1940–1997 from the inner Newfoundland Shelf averaged over the water column and filtered to remove the seasonal signal. The horizontal reference lines in Figs. 10*b*–10*d* are means.



caused directly by the fishery (the first prediction of the shift hypothesis). In the fall of 1990, cod were relatively abundant in northern 3K and 2J on the Hamilton Bank, as was evident from the fall survey. However, cod aggregations could not be located in the same region a few months later at the onset of the winter fishery (very low CPUE) but were readily located further south. It might be argued that declining effort in the north in 1991 disallows the conclusion that few fish were there. However, fishers argue that effort in 2J was sufficient to locate any major aggregations (J. Kelly, former trawler captain, Little Bay, Placentia Bay, personal communication), and evidence shows that by the late-1980s, this fleet had extraordinary knowledge of fish movements and locations, in fact targeting the remaining aggregations with great acuity (Rose and Kulka 1999). Moreover, the very high CPUE in 3K and northern 3L in winter 1991 is consistent with cod having shifted south by this time. Southern shifts have been previously reported. Travin and Pechenik (1963) reported that "under definite conditions the cod inhabiting the Labrador area spawn considerably farther southward (3K and 3L)." Wroblewski et al. (1995) reported southerly movements along the edge of the continental shelf in early winter. Consistent with such a shift, in the spring of 1991, cod were abundant in the south (3K and 3L). Seasonal movements southward are also consistent with the spatial and temporal returns of fish tagged in the southern range in 1990 and 1991 (Rose 1993). However, seasonal migrations ceased by the fall of 1992 and cannot account for the continued reduction of fish in the north. Higher mortality rates in the northern than in the southern populations fished in the south during the early 1990s could account for the quicker decline in the north. However, selection of northern fish by these fisheries seems unlikely, especially as the northern fish are smaller at age.

Large-scale distribution changes are also evident between 1992 and 1994, when there was no fishery (the offshore fishery ceased in February 1992, and a full moratorium on fishing within the Canadian EEZ was implemented July 2,

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1992). Despite the moratorium, the biomass of cod utilizing the Bonavista corridor declined from approximately 100 000 to a few thousand tonnes from 1992 to 1994. Cod were observed moving further eastward from the corridor along the north cape of the Grand Bank in May of 1991. Following that observation, relatively large numbers of cod, indistinguishable from those monitored in 1992 in terms of age and size, were located approximately 100 km to the east in the winter and spring of 1993 (outside the EEZ) at the same time that fish disappeared in the Bonavista corridor. The most likely explanation for these facts is that the fish remaining in the Bonavista corridor in 1992 migrated from that region by the spring of 1993 and that a substantial portion of them moved further to the east and outside the EEZ. An alternative explanation is a local and natural mass mortality for which we have no evidence.

Our data also indicate that cod densities and abundance increased in the early 1990s in the southern range, even though stock levels were declining (the second prediction of the shift hypothesis). All available data sources exhibited this trend, including the DFO fall survey, the winter fishery, the spring acoustic/trawl research, and the inshore summer fishery. The strength of this conclusion is to be drawn not from any single survey or data source, as all have their biases, but from the consistency of the conglomerate of evidence from such diverse sources. The concentration of cod that occurred in the early 1990s has been termed "hyperaggregation," in which local density not only remains stable but increases as biomass declines (Rose and Kulka 1999). These findings are consistent with the existence in the Bonavista corridor of a dense aggregation of cod measuring approximately 37 by 56 km by 20–100 m deep in June 1990 (Rose 1993) that reformed, albeit in declining volume, each winter–spring from 1990 to 1992. Although the historic record indicates that the Bonavista corridor formed part of the annual cod migration route, the aggregation of such a large number of fish in 1990 (approximately 500 000 000) was unique, at least for the period 1983–1994. Examination of the acoustic record (comparable echograms from 1983 to 1994) indicated that nothing comparable with the 1990 aggregation had been observed previously. There is additional evidence that such a southerly concentration was atypical of earlier periods. Foreign fisheries enjoyed their highest catches and CPUE in the north in the 1950s and 1960s (Travin and Pechenik 1963), and the Canadian fisheries in the late 1970s and early 1980s also had higher CPUE in the north. Why would unrestricted and profit-driven fisheries, with little or no competition among vessels (these were cooperative fleets and there is little indication of a relationship between effort and CPUE in 3L), steam over large and dense aggregations to reach more distant grounds? And why would CPUE be lower in the south than in the north historically and then increase to record levels in the south in the early 1990s, at a time of concentration of effort and a major stock decline? Finally, had there been no distribution shift, the higher fishing effort in the south would have been expected to reduce the southern fish first, not last, as is evident in all available data.

Patterns in the coastal fisheries are also consistent with a distribution shift beginning in the mid- to late 1980s. Relative availability to the southern fisheries increased by an order of magnitude while decreasing in the north, at a time when overall abundance was declining. Of note, trap catches in the south (3L) in 1990 were the highest since statistics were available (1975), while in the more northerly zones, trap catches were very low and declining. Again in 1991, trap catches were relatively high in the southern region and virtually nil to the north. Substantial increases in effort in the trap fisheries almost certainly occurred during this period (e.g., Hutchings and Myers 1994), which may tend to confound the relationship between trap catch and abundance (Rose 1992). However, the order of magnitude increase in availability within a few years is unlikely to be solely an artifact of increases in effort. The record catches in the early 1990s in the south are more likely attributable to changes in distribution and migration patterns.

The offshore fisheries showed patterns similar to those of the inshore fisheries. During the early 1990s, the southern offshore fisheries, both Canadian and foreign, maintained and even increased their CPUE and catches. For the Canadian fisheries, CPUE was maintained because increasing densities of fish were aggregating on southern fishing grounds (Rose and Kulka 1999). The Canadian fleet still attempted to fish in the northern areas in the late 1980s and early 1990s, largely as a result of government regulations, but CPUE was very low. Of note, the Spanish trawler fleet arrived on the nose of the Grand Bank in 1990. This fleet and the rest of the foreign fishery reported record catches of cod in 1991 (>50 000 t reported). It is difficult to conceive how such catches could have occurred without southern movements of cod to this fishing area, as has been demonstrated for the better studied fishing grounds within the Canadian EEZ (Rose and Kulka 1999).

We also found more direct evidence of real changes in cod distribution in the early 1990s (the third prediction of the shift hypothesis). The winter pattern of antifreeze production in cod captured in the south was consistent for over a decade until 1990. However, cod captured in the same region in 1991 had a much greater capacity to produce antifreeze than any group previously studied. This shift was abrupt and unexpected, and the observed elevation in cold hardiness continued through 1994. Overwintering temperatures were no more severe in the years of exceptional antifreeze production than in the preceding 10 years and thus cannot be invoked to explain these observations.

Population differences in antifreeze production capacity have been found in several northern teleost species that have a wide geographic distribution and a greater need for cold protection at high latitudes. Both winter flounder (*Pleuronectes americanus*) and ocean pout (*Macrozoarces americanus*) from the Newfoundland region are capable of developing significantly higher antifreeze levels than fish of the same species from further south (Fletcher et al. 1985*a*, 1985*b*). This phenomenon has recently been described in the Atlantic cod. Juvenile Atlantic cod collected from a northerly site (the tip of Newfoundland's Great Northern Peninsula) developed TH values approximately 50% higher than juvenile cod collected from three bays along the north coast of Newfoundland when held under identical conditions over a winter period (Goddard et al. 1999). TH values between adults and juveniles cannot be directly compared, since juvenile cod are known to have a greater antifreeze production

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capacity than adults (Kao and Fletcher 1988; Goddard et al. 1992). However, the postshift adult cod collected in the southerly region between 1991 and 1994 developed TH levels considerably more than 60% higher than the adults collected from the same area between 1980 and 1990. Thus, the elevation of antifreeze production capacity of the postshift adults over that of the preshift adults more than equals the elevation of antifreeze production capacity of northern juveniles over juvenile cod from more southerly areas.

These data lead us to conclude that the abrupt upward shift (1991–1992) in the antifreeze production capacity of cod sampled in the southern region between 1980 and 1995 was due to an influx into the area of cod originating further north from an area demanding even greater freeze protection for survival. Since the shift was first reported in the winter of 1991–1992, this suggests that some northern fish had moved into southern waters by the summer of 1991.

Meristic evidence is also consistent with the hypothesis that northern fish were present in their southern range in the early 1990s. The vertebral count is formed during early development and is temperature dependent. Slower growing northern fish have a higher count. Postolakii (1963) and Templeman (1981) demonstrated a difference between northern and southern fish in this stock. However, in 1994, there was no difference among the northern and southern areas, and southern fish had vertebral counts identical to those of northern fish in historical data. Although this change might also be explained by environmental changes between historical and recent times, the vertebral data are consistent with fish movement from north to south.

The rather abrupt decline in mean weight-at-age of cod in the south (3L) in 1991 also supports the prediction that northern fish shifted to southern waters. The mean weight of southern fish of ages 5–7, which formed the bulk (>80%) of the extant migrating biomass (cod of the northern stock begin large scale migrations at ages 4–5 (Rose 1993)), declined to near the level of northern fish of the same age by 1992. It is important to note that the decline in weight-at-age was not a consequence of declining condition in southern fish. Condition of 3L fish remained stable from 1981 to 1992 (Taggart et al. 1994). We can think of no alternative explanation for the decline in weight-at-age other than an incursion of smaller fish in equal condition. The condition of fish that remained in 2J declined after 1989 (Taggart et al. 1994), which suggests that environmental conditions worsened in the north.

Recent studies suggest that there may be genetic differences between cod resident from the Bonavista corridor northward and those on the Grand Bank (Bentzen et al. 1996; Ruzzante et al. 1996, 1998). Unfortunately, the data used in these studies were mostly from juvenile cod and not from adults of separate spawning groups. It is noteworthy that Bentzen et al. (1996) concluded that their results indicated a different stock structure than suggested by deYoung and Rose (1993). However, we interpret the results of Bentzen et al. (1996) and Ruzzante et al. (1996, 1999) as broadly supportive of deYoung and Rose (1993), who hypothesized the existence of a geographic cline of populations from Labrador to the northern area of the Grand Bank. Of note, the known migration routes of the northern cod do not cross onto the Grand Bank except at the southern margins of the Bonavista corridor in the north cape region (Rose 1993; Colbourne et al. 1997*b*). Hence, movements from southern Labrador to the north cape of the Grand Bank are within the stock range of northern cod, and no major genetic differences are to be expected. However, we suggest that the north cape may be a seasonal or episodic mixing area for northern and Grand Bank cod, which differs somewhat from Ruzzante et al. (1999). It is noteworthy that cod collected on the nose of the Grand Bank in the winter of 1993 differed from other southern cod in allele frequencies at one locus but did not differ from fish sampled further north (Bentzen et al. 1996). These fish were subsequently removed from analyses conducted by Bentzen et al. (1996) because they did not resemble other Grand Bank cod. The location of genetically northern-type fish on the northeast corner of the Grand Bank is consistent with the hypothesis that northern cod were present in the southern ranges, and to the nose of the Grand Bank, in the early 1990s.

Following our interpretation of observations from the 1980s and early 1990s, the arguments put forth by Hutchings (1996) that no large-scale changes in distribution or migration patterns occurred during the early 1990s require examination. Tagging studies cited by Hutchings (1996) that purport not to support movement (Myers and Cadigan 1995) cannot resolve the shift hypothesis. Unfortunately, no tagging was conducted in the northern area (2J) immediately before or during the years when movement is thought to have occurred (see Taggart et al. (1995) for a synopsis of most of the tagging data). Moreover, the use of fish tagged in 3K as "northern" in opposition to northern 3L as "southern" (Myers and Cadigan 1995) is unwarranted because the main migration route of the northeast shelf component of the stock crosses the 3K–3L boundary (Rose 1993; Wroblewski et al. 1995; Colbourne et al. 1997*b*). Hutchings (1996) also argued that the presence of northern cod in the north during the fall of 1990 and 1991 (e.g., Fig. 4) is evidence against the shift hypothesis (as did Lilly 1994). However, their interpretations are based entirely on fall data. Seasonal data show that a transient northern presence in fall was likely a residual of the migration cycle. The seminal point is that these fish did not stay in the north for the critical period of overwintering and spawning, as once they may have. Moreover, by 1992, even a transient presence in the north was not evident.

Hutchings (1996) also argued, as we have, that size-at-age of older migratory fish in the south would be expected to have declined somewhat in the early 1990s if northern fish had shifted south. However, Hutchings (1996) used only 2 years of data from the DFO fall survey to conclude that size-at-age did not decline in the southern zone (3L) in 1989 or 1990. A more complete analysis indicated that weight-at-age declined by approximately 25% in the south, to levels nearer those of northern fish, in 1991 and 1992. Hence, these results are fully consistent with a shift of lesser weight-at-age fish from the northern to the southern region in the early 1990s. It is noteworthy that the decline was not apparent until after 1990, although the shift may have begun a year or two earlier. However, up until 1991, the component of the fall migration that includes a northern route back to the wintering grounds was still much in evidence. Hence, although a winter shift may have begun in the late 1980s, its full impact

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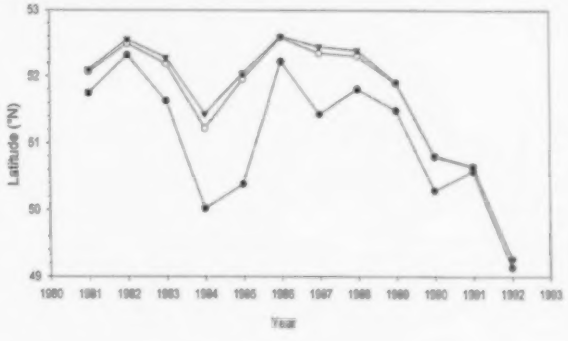
was not to be observed in the fall survey, or the spring surveys, until the early 1990s. For similar reasons, the abrupt change in antifreeze production may not have been evident until 1991 in the southern zone.

Some of the criticisms of Hutchings (1996) address uncertainties about the cause of the shift, in particular related to environmental hypotheses. Rose et al. (1994), employing both survey and fishery data, showed that on average, cod were located further north in warm years and south in cold years. Hutchings (1996) claimed that these findings were dependent on the inclusion of fish from the northern Grand Bank, which may or may not be a component of the northern stock (we agree that this is uncertain). However, reanalyses of subsets of the same data from which were excised an increasing proportion of the southerly range up to 47.5°N, well to the north of the contested fish, did not change the overall interannual distribution pattern (Fig. 11). Moreover, the offshore fishery data mirror these survey data (Rose et al. 1994). There is also confirmatory evidence from the inshore fisheries, as southern trap catches have been invariably high when the cold intermediate layer anomaly (Colbourne et al. 1997*a*) is positive. Hence, survey and fishery data consistently suggest that cod are more southerly distributed in colder years and more northerly distributed in warmer years. We believe analyses of mean latitudes to be meaningful and to support the shift hypothesis, in contradiction of Hutchings and Myers (1994) and Hutchings (1996).

Fig. 11. Mean latitude of northern cod distribution (weighted by standardized CPUE) for 1981–1992 under various southern boundary conditions: >46°N (solid circles), >47°N (open circles), >47.5°N (inverted triangles).



What caused the shift?

A first hypothesis is that the shift was caused by abiotic environmental change. Latitudinal shifts do correlate with sea temperatures (Rose et al. 1994). Moreover, the NAO reached its peak value in the 60-year series in 1989, the year that the recent major shift began in earnest. In the cold years of the early 1990s that followed, cod were located in the warmest waters available during the winter and spring. For example, mean water temperatures where cod aggregated in the outer Bonavista corridor in June 1991, the coldest year on record, were above 3.5°C (0.5°C above the historical average for that area). Nevertheless, there are difficulties in concluding that cod simply moved either to avoid cold conditions (Lilly 1994) or to seek out warmer conditions. Warm waters (2–4°C) exist at depths >300 m in all Newfoundland regions even in the coldest years (although the distribution of these temperatures may be contracted). It is also uncertain as to what temperatures cod actually experience over a year and how they might react to differing seasonal temperature regimes. Seasonal temperature variability is much greater than interannual variability, except near the bottom (Petrie et al. 1991). Hence, cod migrating near bottom may be most influenced by the annual signal. In perhaps the best attempt thus far to resolve these complexities, Colbourne et al. (1997*b*) showed that during a single migration cycle, cod may experience temperatures that average a full degree colder in cold than in warm years. The effects that such differences may have on cod distribution are uncertain. deYoung and Rose (1993) hypothesized that cod might integrate thermal conditions over the seasons, and hence respond to the annual signal. If true, broad-scale measures of the thermal state of the ecosystem, or broad-scale indices of ocean climate such as the NAO, may be more valid than single measures of where cod are at a particular time (during a survey) as indicators of annual distribution. Such a relationship could explain the "paradox" of a relationship between temperature and cod distribution in this stock (deYoung and Rose 1993) and why north–south distribution correlates with broad-scale thermal indices (Rose et al. 1994). In conclusion, although the mechanism remains opaque, variations in ocean climate almost certainly influence the latitudinal distribution of northern cod.

A second hypothesis is that northern cod shifted their distribution in response to changes in the biotic environment, in particular the distribution of capelin, their chief prey. A major decline of capelin off Labrador occurred in 1989, just before the southerly shift in cod (Lilly 1994; Carscadden and Nakashima 1997). During this period, capelin moved east to the Flemish Cap, well outside the Canadian EEZ, and perhaps south as far as the Scotian Shelf (Frank et al. 1996). Within the known range of the northern cod, capelin concentrated during the early 1990s on the north cape of the Grand Bank and just south of the Bonavista corridor (see Lilly 1994), in areas adjacent to those where cod aggregated. The capelin shift has also been linked to cooling ocean conditions (Carscadden and Nakashima 1997). Hence, a prey shift is a likely mechanism linking a shift in cod to changes in ocean climate.

A third hypothesis is that the fisheries of the past 40 years have influenced distribution patterns in less direct ways than simply fishing down concentrations. There are several possible fishery-related effects on stock dynamics and behavior that could underlie a distribution shift. MacCall (1990) proposed a "basin model" in which density dependence leads to range contraction to "favored" habitat during stock decline. deYoung and Rose (1993) used this model as a basis for their hypothesis on distribution shift but modified it to include environmental change as a cause of range contraction. Cod have been reported to modify their thermal associations as stock abundance changes (Swain and Wade 1993). Hence, because of abundance declines (MacCall 1990), environmental effects (deYoung and Rose 1993), or an interaction between these factors, distribution may contract to favored habitat. However, for migratory stocks such as northern cod that spawn and feed in disparate regions, the identification of

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a single favored habitat is problematic. For example, the Bonavista corridor, the area where northern cod last congregated in the early 1990s, was likely near optimal in terms of thermal conditions (warm) and adjacency to prey (capelin). However, this same area was likely poor habitat in terms of spawning. deYoung and Rose (1993) correctly predicted that southern spawning in 1991 and 1992 would produce poor recruitment. Moreover, if the widespread historical population structure of the northern cod was fundamental to productivity (deYoung and Rose 1993), then the long-term effects of decades of over-fishing may not only have been to directly reduce population levels, but also to increase the latent productivity of this stock by reducing the potential for diversity in spawning distribution and migration patterns.

The fisheries almost certainly resulted in a collapsed age structure. Historically, fish 10–20 years of age were a significant component of the northern cod (see data in Bishop et al. 1993). As late as 1990, there were substantial numbers of age 8+ fish, but by 1992, there were few fish >6 years of age. Such a reduction, combined with the reduction in biomass by a factor of 5 during these years, led Rose (1993) and Rose et al. (1995) to speculate that migration patterns were changing as a consequence of density- and age-dependent effects. In brief, with fewer fish, there would be less impetus to migrate, and with fewer older fish, the social transmission of the routes might cease, and migration might occur imprecisely or not at all (Rose et al. 1995). These predictions are consistent with recent events, as the migration ceased in 1993 and has not been evident since.

The fishery could also have altered habitat. However, we have no data on the northern cod habitat with which to address this hypothesis.

In summary, there is evidence that ocean climate change, shifting prey distributions, and density- and age-dependent population responses resulting from the fishery all played a significant and interactive role in the changes in distribution and migration patterns observed in the northern cod in the late 1980s and early 1990s. This complex situation underscores the need for a more comprehensive "ecosystem" approach to fisheries.

Fishery and the environment: what caused the decline?

Large-scale unsustainable fishing practices began in the 1950s and decimated key northern components of the once abundant Labrador fishery. It is our view that the northern cod never recovered from this over-fishing. Three decades later, there is little doubt that fishing mortality again was unsustainable during the late 1980s and early 1990s and led to more widespread population collapse (Hutchings and Myers 1994; Sinclair and Murawski 1997). The question is whether environmental factors played a role in the collapse and hence may be expected to play a role in the future. We have shown that the decline of the northern cod was not simply a fishing down of concentrations from north to south, as argued by Hutchings and Myers (1994, and several other papers by the same authors). It appears near certain that major environmental changes and several ecological anomalies took place during the period of cod decline (Drinkwater and Mountain 1997; Sinclair and Murawski 1997) and that these contributed substantially to the decline in the northern cod. The southward shift in northern cod led to increased vulnerability to fishing (Rose et al. 1994) and to reduced recruitment (deYoung and Rose 1993). The environmental changes affected many species other than cod. Capelin exhibited slow growth and delayed spawning and shifted southwards beginning in the late 1980s (Frank et al. 1996; Carscadden and Nakashima 1997). These changes have been attributed to the cooler waters of the 1990s (Carscadden and Nakashima 1997). Montevecchi and Myers (1997) documented an abrupt change in food for gannets, from Atlantic mackerel (*Scomber scombrus*) to capelin, on Funk Island off the northeast coast of Newfoundland in 1990. Atlantic salmon (*Salmo salar*) exhibited changed migration patterns in the early 1990s (Narayanan et al. 1995). Other species, such as American plaice (*Hippoglossoides platessoides*), declined under low exploitation (Bowering et al. 1997). Species as diverse as marine butterflies (*Oikopleura* spp.) and Arctic cod, whose historical ranges were much more northerly, became widely distributed in the southern areas in the early 1990s (e.g., Drinkwater and Mountain 1997). Pandalid shrimp and snow crab populations have surpassed any previously observed levels. It is difficult to find any aspect of the northern cod ecosystem that did not change in the late 1980s and early 1990s.

In conclusion, we think it unlikely that all of the changes in the northern cod ecosystem, or in the cod populations themselves, can be attributed in total to the fisheries. Large fluctuations in the cod stocks would likely occur with no fisheries at all, as reported from 1710 to 1720 (Innes 1940). Nevertheless, there is little doubt that the catastrophic and sustained declines in cod evident over the past 40 years would not have occurred without the fisheries, and the effects of these fisheries have been manifest not only in the cod populations but throughout the ecosystem. The key question is how persistent these changes will be, and are some, indeed, irreversible.

After the moratorium: did the foreign fishery play a role?

The moratorium on the fishery in the Canadian EEZ halted neither the changes in distribution patterns nor the decline in northern cod abundance. We propose that the two factors are related, as for the earlier period from the late 1980s to 1992. We have presented evidence that southerly movements along the northeast shelf may have been extended along the north cape of the Grand Bank in the early 1990s, especially after 1992. Such movement is consistent with the observed presence of large aggregations of cod outside the EEZ in the winter and spring of 1993. It is noteworthy that beginning in 1990 and continuing after the Canadian moratorium in July 1992, more than 50 trawlers from EU countries fished on the nose of the Grand Bank. This fishery could account for the disappearance of over 2000 tagged fish, and their aggregation, that were present on the north cape of the Grand Bank in the spring of 1991, and moving east toward the boundary of the EEZ, and for the disappearance of the extant aggregations located outside the EEZ in 1993. It is also likely that this fishery contributed to the disappearance of the fish (approximately 100 000 t) present in the Bonavista corridor in 1992, if these fish moved southeastward. Data in Kulka et al. (1995) show a progressive shift in the areas of high catch rates of the Canadian fleet to

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the southeast beginning in the late 1980s, such that by 1992, the largest fishing ground was adjacent to the EEZ limit. The reported catch of the foreign fleet was approximately 51 000 t in 1991. The true removals will likely never be known, but it is clear that despite the steep decline in stock abundance, a minimum of 51 000 t was available in the small area of the nose of the Grand Bank (approximately 10 000 km^2). Such availability implies a mean density approaching 0.01 fish·m^{-2} over the full area, if the fishery caught every fish there (and much higher local densities if a more typically heterogenous distribution pattern is assumed (e.g., Rose and Kulka 1999)). Given that such densities are comparable with those measured in the Bonavista corridor in the early 1990s (Rose 1993), it is implausible that they represent anything but an active movement to and concentration of fish on the nose of the Grand Bank. The EU (Spanish) fleet was well positioned for fishing southward-migrating cod. We conclude that distribution shifts led to continued fishing after the moratorium in the Canadian EEZ in 1992 and continued population decline.

The future

Will the northern cod rebuild? How long will it take and how will rebuilding occur? Recruitment of juveniles across the former range is the key to rebuilding. Over the past decade, recruitment has been generally poor. The southern spawning distributions of 1991–1993 generated almost nil recruitment (Anderson and Dalley 1997), and despite a slight improvement in 1994, more recent recruitment has been poor (Lilly et al. 1999). Thus far, the more abundant inshore spawning (Rose 1996, unpublished data) appears not to have repopulated the larger shelf zone (Anderson and Dalley 1997). In contrast, a very limited northern shelf spawning (G.A. Rose, unpublished data) in 1994 resulted in an immediate increase in the numbers of juveniles on the shelf (Anderson and Dalley 1997). Hence, we conclude that rebuilding the northern shelf spawning groups is the key to the rebuilding of the full stock.

Will the northern spawning grounds be recolonized or must all rebuilding occur locally? At present, we cannot definitively answer this question. However, cod off northern Labrador (2GH) have not rebuilt since their decline four decades ago in the 1960s. The reasons for this are not known. But the question arises as to why 2J fish did not recolonize these more northerly areas. There are several possible explanations: (*i*) abiotic or biotic environmental conditions were unfavorable, (*ii*) 2J fish never attained some abundance threshold necessary for emigration (2J was heavily fished during this period, and this fishery spilled over the 2J–2H boundary in the late 1970s, which suggests that 2J fish may have attempted to move north), and (*iii*) genetic or behavioral constraints made recolonization problematic. It may be important that high antifreeze producers, and presumably northern fish, were still present off southern Newfoundland in the summer of 1994 (the last year of sampling). This suggests that, in terms of cold tolerance, the genetic or phenotypic diversity necessary for northern recolonization has not been eliminated.

The rebuilding of the northern cod was predicted to take decades, based on the distributions model of deYoung and Rose (1993). Alternatively, projections of rebuilding schedules based on nonecological population models, formulated on the notion that stocks will simply increase at some fixed rate when fishing stops (e.g., Myers et al. 1997), indicated much faster rebuilding. For example, projections by Roughgarden and Smith (1996) based on such theory indicate that northern cod abundance should be over 1.5×10^6 t by 1997! The reality is closer to an order of magnitude lower (Lilly et al. 1999). Similar projections made by Myers et al. (1997) based on a lower growth rate are more realistic but still overestimate observed population recovery. This disparity suggests that factors other than current population size and parameters internal to populations are important to the rebuilding process and that external "ecosystem" factors play a role in population growth. Behavioral changes at low population sizes may also complicate any simplistic view of stock rebuilding. Based on the present studies, we cannot forecast a timetable for rebuilding, but we can forecast steps thought to be diagnostic of the stage of rebuilding. In stage 1, holding to the theory that northern spawning drives population abundance in the northern stock, abiotic and biotic conditions in the north must revert to favorable states for cod. Of importance are warming conditions and the return and reestablishment of capelin as a major food source. It is noteworthy that oceanic conditions off Labrador have returned to normal since 1996 (Colbourne et al. 1997*a*), and a resurgence of capelin off Labrador has recently been observed (R. O'Driscoll, Fisheries and Marine Institute, St. John's, Nfld., unpublished data). In stage 2, adult cod reverse the southerly shift back to the north and in time reform large (>100 000 t) spawning aggregations. It follows that if rebuilding of the historic stock complex is an objective, both cod and capelin stocks must increase in numbers (and age) so that emigration from southern regions is more likely to occur. Reseeding of juveniles from Greenland waters is also possible, but at present unlikely as a result of low stock levels there (Dickson and Brander 1994). In stage 3, recruitment of juveniles across the shelf will begin. We are currently at stage 1. There is precedent for such staged recoveries of large marine fish stocks. The Norwegian spring-spawning herring, devastated by overfishing in the 1970s, reestablished a full migratory life cycle in stops and starts from a much reduced area inhabited for almost three decades (Dragesund et al. 1977). Hence, the adaptive migratory behavior of the northern cod, which in the early 1990s caused major shifts in distribution and amplified the effects of overfishing on the undoing of this stock, may also be its salvation.

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References

Anderson, J.T., and Dalley, E.L. 1997. Spawning and year-class strength of northern cod (*Gadus morhua*) as measured by pelagic juvenile cod surveys, 1991–1994. Can. J. Fish. Aquat. Sci. **54**(Suppl. 1): 158–167.

Atkinson, D.B., Rose, G.A., Murphy, E.F., and Bishop, C.A. 1997. Distribution changes and abundance of northern cod (*Gadus morhua*), 1981–1993. Can. J. Fish. Aquat. Sci. **54**(Suppl. 1): 132–138.

Bentzen, P., Taggart, C.T., Ruzzante, D.E., and Cook, D. 1996. Microsatellite polymorphism and the population structure of Atlantic cod (*Gadus morhua*) in the northwest Atlantic. Can. J. Fish. Aquat. Sci. **53**: 2706–2721.

Bishop, C.A., Murphy, E.F., Davis, M.B., Baird, J.W., and Rose, G.A. 1993. An assessment of the cod stock in NAFO divisions 2J+3KL. NAFO SCR Doc. 93/96.

Bowering, W.R., Morgan, M.J., and Brodie, W.B. 1997. Changes in the population of American plaice (*Hippoglossoides platessoides*) off Labrador and northeastern Newfoundland: a collapsing stock with low exploitation. Fish. Res. **30**: 199–216.

Brander, K.M. 1994. Patterns of distribution, spawning, and growth in North Atlantic cod: the utility of inter-regional comparisons. ICES J. Mar. Sci. Symp. **198**: 406–413.

Brander, K.M. 1997. Effects of climate change on cod (*Gadus morhua*) stocks. *In* Global warming: implications for freshwater and marine fish. *Edited by* C. Wood and G. McDonald. Cambridge University Press, Cambridge, U.K. p. 320.

Carscadden, J., and Nakashima, B.S. 1997. Abundance and changes in distribution, biology, and behavior of capelin in response to cooler waters of the 1990s. *In* Forage Fishes in Marine Ecosystems: Proceedings of the International Symposium on the Role of Forage Fishes in Marine Ecosystems. Univ. Alaska Sea Grant College Program Rep. 97-01. pp. 457–468.

Colbourne, E., deYoung, B., Narayanan, S., and Helbig, J. 1997*a*. Comparison of hydrography and circulation on the Newfoundland Shelf during 1990–1993 with the long-term mean. Can. J. Fish. Aquat. Sci. **54**(Suppl. 1): 68–80.

Colbourne, E., deYoung, B., and Rose, G.A. 1997*b*. Environmental analysis of Atlantic cod (*Gadus morha*) migration in relation to the seasonal variations on the Northeast Newfoundland Shelf. Can. J. Fish. Aquat. Sci. **54**(Suppl. 1): 149–157.

Crittenden, R.N., Thomas, G.L., Marino, D.A., and Thorne, R.E. 1988. A weighted duration-in-beam estimator for the volume sampled by a quantitative echosounder. Can. J. Fish. Aquat. Sci. **45**: 1249–1256.

Davies, P.L., and Hew, C.L. 1990. Biochemistry of fish antifreeze proteins. FASEB J. **4**: 2460–2468.

DeVries, A.L. 1988. The role of antifreeze glycopeptides and peptides in the freezing avoidance of Antarctic fishes. Comp. Biochem. Physiol. B, Comp. Biochem. **90**: 611–621.

deYoung, B., and Rose, G.A. 1993. On recruitment and distribution of Atlantic cod (*Gadus morhua*) off Newfoundland. Can. J. Fish. Aquat. Sci. **50**: 2729–2741.

Dickson, R.R., and Brander, K.M. 1994. Effects of a changing windfield on cod stocks of the North Atlantic. ICES Mar. Sci. Symp. **198**: 271–279. (Extended abstract.)

Dickson, R.R., Lazier, J., Meinke, J., Rhines, P., and Swift, J. 1996. Long term changes in the convective activity of the North Atlantic. Prog. Oceanogr. **38**: 241–295.

Doubleday, W.G., and Rivard, D. 1981. Bottom trawl surveys. Can. Spec. Publ. Fish. Aquat. Sci. No. 58.

Dragesund, O., Hamre, J., and Ulltang, O. 1977. Biology and population dynamics of the Norwegian spring-spawning herring. Rapp. P.-v. Réun. Cons. Int. Explor. Mer, **177**: 43–71.

Drinkwater, K.F., and Mountain, D.G. 1997. Climate and oceanography. *In* Northwest Atlantic groundfish: perspectives on a fishery collapse. *Edited by* J. Boreman, B.S. Nakashima, J.A. Wilson, and R.L. Kendall. American Fisheries Society, Bethesda, Md. pp. 3–25.

Fahrig, L., Pope, S.E., Henein, K.M., and Rose, G.A. 1998. Relative effects of trap versus trawl fisheries on population dynamics of the northern cod (*Gadus morhua*) stock. Can. J. Fish. Aquat. Sci. **55**: 76–85.

Fletcher, G.L., Haya, K., King, M.J., and Reisman, H.M. 1985*a*. Annual antifreeze cycles in Newfoundland, New Brunswick, and Long Island winter flounder *Pseudopleuronectes americanus*. Mar. Ecol. Prog. Ser. **21**: 205–212.

Fletcher, G.L., Hew, C.L., Li, X., Haya, K., and Kao, M.H. 1985*b*. Year-round presence of high levels of plasma antifreeze peptides in a temperate fish, ocean pout (*Macrozoarces americanus*). Can. J. Zool. **63**: 488–493.

Fletcher, G.L., King, M.J., and Kao, M.H. 1987. Low temperature regulation of antifreeze glycopeptide levels in Atlantic cod (*Gadus morhua*). Can. J. Zool. **65**: 227–233.

Foote, K.G., Knudsen, H.P., Vestnes, G., Maclennan, D.N., and Simmonds, E.J. 1987. ICES Coop. Res. Rep. No. 144.

Frank, K.T., Carscadden, J.E., and Simon, J.E. 1996. Recent excursions of capelin (*Mallotus villosus*) to the Scotian Shelf and Flemish Cap during anomalous hydrographic conditions. Can. J. Fish. Aquat. Sci. **53**: 1473–1486.

Goddard, S.V., Kao, M.H., and Fletcher, G.L. 1992. Antifreeze production, freeze resistance, and overwintering of juvenile northern Atlantic cod (*Gadus morhua*). Can. J. Fish. Aquat. Sci. **49**: 516–522.

Goddard, S.V., Kao, M.H., and Fletcher, G.L. 1999. Population differences in antifreeze production cycles of juvenile Atlantic cod (*Gadus morhua*) reflect adaptations to overwintering environment. Can. J. Fish. Aquat. Sci. **56**: 1991–1999.

Gomes, M.C., Haedrich, R.L., and Villagarcia, M.G. 1995. Spatial and temporal changes in the groundfish assemblages on the Northeast Newfoundland/Labrador Shelf, Northwest Atlantic, 1978–1991. Fish. Oceanogr. **4**: 85–101.

Harris, L. 1990. Independent review of the state of the northern cod stock. DFO Rep. No. 4379. Department of Fisheries and Oceans, Ottawa, Ont.

Hutchings, J.A. 1996. Spatial and temporal variation in the density of northern cod and a review of hypotheses for the stock's collapse. Can. J. Fish. Aquat. Sci. **53**: 943–962.

Hutchings, J.A., and Myers, R.A. 1994. What can be learned from the collapse of a renewable resource? Atlantic Cod, *Gadus morhua*, of Newfoundland and Labrador. Can. J. Fish. Aquat. Sci. **51**: 2126–2146.

Innes, H.A. 1940. The cod fisheries: the history of an international economy. University of Toronto Press, Toronto, Ont.

Jennings, S., and Kaiser, M.J. 1998. The effects of fishing on marine ecosystems. Adv. Mar. Biol. **34**: 201–352.

Kao, M.H., and Fletcher, G.L. 1988. Juvenile Atlantic cod (*Gadus morhua*) can be more freeze resistant than adults. Can. J. Fish. Aquat. Sci. **45**: 902–905.

Kao, M.H., Fletcher, G.L., Wang, N.C., and Hew, C.L. 1986. The relationship between molecular weight and antifreeze polypeptide activity in marine fish. Can. J. Zool. **64**: 578–582.

Krohn, M., Reidy, S., and Kerr, S. 1997. Bioenergetic analysis of the effects of temperature and prey availability on growth and condition of northern cod (*Gadus morhua*). Can. J. Fish. Aquat. Sci. **54**(Suppl. 1): 113–121.

© 2000 NRC Canada

Kulka, D.W., and Firth, J.R. 1987. Observer program training manual — Newfoundland region. Can.Tech. Rep. Fish. Aquat. Sci. No. 1355.

Kulka, D.W., Wroblewski, J.S., and Narayanan, S. 1995. Interannual patterns in the winter distribution and recent changes and movements of northern Atlantic cod (*Gadus morhua*) on the Newfoundland–Labrador shelf. ICES J. Mar. Sci. **52**: 889–902.

Kulka, D.W., Pinhorn, A.T., Halliday, R.G., Pitcher, D., and Stansbury, D. 1996. Accounting for changes in spatial distribution of groundfish when estimating abundance from commercial fishing data. Fish. Res. **28**: 321–342.

Larkin, P. 1996. Concepts and issues in marine ecosystem management. Rev. Fish Biol. Fish. **6**: 139–164.

Lawson, G.L., and Rose, G.A. 1999. The importance of detectability to acoustic surveys of semi-demersal fish. ICES J. Mar. Sci. **56**: 370–380.

Lilly, G.R. 1994. Predation by Atlantic cod on capelin on the southern Labrador and Northeast Newfoundland shelves during a period of changing spatial distributions. ICES J. Mar. Sci. Symp. **198**: 600–611.

Lilly, G.R., Shelton, P.A., Brattey, J., Cadigan, N., Murphy, E.F., and Stansbury, D.E. 1999. An assessment of the cod stock in NAFO Divisions 2J+3KL. DFO Can. Stock Assess. Secretariat Res. Doc. 99/42.

MacCall, A.D. 1990. Dynamic geography of marine fish populations. Washington Sea Grant Program, University of Washington Press, Seattle, Wash.

MacLennan, D.N., and Simmonds, E.J. 1992. Fisheries acoustics. Chapman and Hall, London, U.K.

Mann, K.H. 1993. Physical oceanography, food chains, and fish stocks: a review. ICES J. Mar. Sci. **50**: 105–119.

Montevecchi, W.A., and R.A. Myers. 1997. Centurial and decadal oceanographic influences on changes in northern gannet populations and diets in the North-west Atlantic: implications for climate change. ICES J. Mar. Sci. **54**: 608–614.

Myers, R.A., and Cadigan, N.G. 1995. Statistical analysis of catch-at-age data with correlated errors. Can. J. Fish. Aquat. Sci. **52**: 1265–1273.

Myers, R.A., Mertz, G., and Fowlow, P.S. 1997. Maximum population growth rates and recovery times for Atlantic cod, *Gadus morhua*. Fish. Bull. U.S. **95**: 762–772.

Myers, R.A., Hutchings, J.A., and Barrowman, N.J. 1996. Hypotheses for the decline of cod in the North Atlantic. Mar. Ecol. Prog. Ser. **138**: 293–308.

Narayanan, S., Carscadden, J., Dempson, J.B., O'Connell, M.F., Prinsenberg, S., Reddin, D.G., and Shackell, N. 1995. Marine climate off Newfoundland and its influence on Atlantic salmon (*Salmo salar*) and capelin (*Mallotus villosus*). Can. Spec. Publ. Fish. Aquat. Sci. No. 121. pp. 461–474.

Parsons, T.R., and Seki, H. 1995. An historical perspective of biological studies in the ocean. Aquat. Living Resour. **8**: 113–122.

Petrie, B., Loader, J., Akenhead, S., and Lazier, J. 1991. Temperature and salinity variability on the eastern Newfoundland shelf: the annual harmonic. Atmos.-Ocean, **29**: 14–36.

Postolakii, A.I. 1963. Biology of the Labrador and Newfoundland cod. *In* Soviet fisheries investigations in the Northwest Atlantic. *Translated by* B. Hershkovitz. U.S. Department of the Interior and the National Science Foundation,Washington, D.C. pp. 338–348.

Rose, G.A. 1992. Indices of total stock biomass in the "northern" and Gulf of St. Lawrence Atlantic cod (*Gadus morhua*) stocks derived from time series analyses of fixed gear (trap) catches. Can. J. Fish. Aquat. Sci. **49**: 202–209.

Rose, G.A. 1993. Cod spawning on a migration highway in the north-west Atlantic. Nature (Lond.), **366**: 458–461.

Rose, G.A. 1996. Cross-shelf distributions of cod in NAFO Divisions 2J3KL in May and June 1995: some preliminary findings of a longer term study. NAFO SCR Doc. 96/57.

Rose, G.A., and Kulka, D.W. 1999. Hyperaggregation of fish and fisheries: how catch-per-unit-effort increased as the northern cod (*Gadus morhua*) declined. Can. J. Fish. Aquat. Sci. **56**(Suppl. 1): 118–127.

Rose, G.A., and Porter, D.R. 1996. Target-strength studies on Atlantic cod (*Gadus morhua*) in Newfoundland waters. ICES J. Mar. Sci. **53**: 259–265.

Rose, G.A., Atkinson, B.A., Baird, J., Bishop, C.A., and Kulka, D.W. 1994. Changes in distribution of Atlantic cod and thermal variations in Newfoundland waters, 1980–1992. ICES J. Mar. Sci. Symp. **198**: 542–552.

Rose, G.A., deYoung, B., and Colbourne, E.B. 1995. Cod (*Gadus morhua* L.) migration speeds and transport relative to currents on the north-east Newfoundland Shelf. ICES J. Mar. Sci. **52**: 903–913.

Roughgarden, J., and Smith, F. 1996. Why fisheries collapse and what to do about it. Proc. Natl. Acad. Sci. U.S.A. **93**: 5078–5083.

Ruzzante, D.E., Taggart, C.T., Cook, D., and Goddard, S. 1996. Genetic differentiation between inshore and offshore Atlantic cod (*Gadus morhua*) off Newfoundland: microsatellite DNA variation and antifreeze level. Can. J. Fish. Aquat. Sci. **53**: 634–645.

Ruzzante, D.E., Taggart, C.T., and Cook, D. 1999. A review of the evidence for genetic structure of cod (*Gadus morhua*) in the Northwest Atlantic and population affinities of larval cod off Newfoundland and the Gulf of St. Lawrence. Fish. Res. **43**: 79–97.

Sinclair, A.F., and Murawski, S.A. 1997. Why have groundfish stocks declined? *In* Northwest Atlantic groundfish: perspectives on a fishery collapse. *Edited by* J. Boreman, B.S. Nakashima, J.A. Wilson, and R.L. Kendall. American Fisheries Society, Bethesda, Md. pp. 71–93.

Swain, D.P., and Wade, E.J. 1993. Density-dependent geographic distribution of Atlantic cod (*Gadus morhua*) in the southern Gulf of St. Lawrence. Can. J. Fish. Aquat. Sci. **50**: 725–733.

Taggart, C.T., Anderson, J., Bishop, C., Colbourne, E., Hutchings, J.A., Lilly, G., Morgan, M.J., Murphy, E., Myers, R.A., Rose, G.A., and Shelton, P. 1994. Overview of cod stocks, biology, and environment in the Northwest Atlantic region of Newfoundland, with emphasis on northern cod. ICES J. Mar. Sci. **198**: 140–157.

Taggart, C.T., Penney, P., Barrowman, N., and George, C. 1995. The 1954–1993 Newfoundland cod-tagging database: statistical summaries and spatial–temporal distributions. Can. Tech. Rep. Fish. Aquat. Sci. No. 2042.

Templeman, W. 1981. Vertebral numbers in Atlantic cod, *Gadus morhua*, of the Newfoundland and adjacent areas, 1947–71, and their use for delineating cod stocks. J. Northwest Atl. Fish. Sci. **2**: 21–45.

Travin, V.I., and Pechenik, L.N. 1963. Soviet fishery investigations and fishing Northwest Atlantic. *In* Soviet fisheries investigations in the Northwest Atlantic. *Translated by* B. Hershkovitz. U.S. Department of the Interior and the National Science Foundation, Washington, D.C. pp. 4–55.

Warren, W.G. 1997. Changes in the within-survey spatio-temporal structure of the northern cod (*Gadus morhua*) population, 1985–1992. Can. J. Fish. Aquat. Sci. **54**(Suppl. 1): 139–148.

Wroblewski, J.S., Kulka, D.W., Narayanan, S., Oake, A., Collier, A., and Mcgrath, B. 1995. Winter distribution and movements of northern Atlantic cod (*Gadus morhua*) along the Newfoundland–Labrador continental shelf edge derived from scientific observations on commercial trawlers. Fish. Oceanogr. **4**: 128–146.

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